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THE DRUG-FASTNESS OF SPIROCHETES TO ARSENIC, MERCURIAL, AND IODIDE COMPOUNDS IN VITRO.

By SEINAI AKATSU, M.D., and HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

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It has been known for some time that in trypanosomiasis the trypanosomes which have survived the first effect of an arsenic germicide, such as atoxyl or arsacetin, offer a greater resistance to a subsequent dose of the same drug. By subjecting the organisms to repeated injections of an arsenic medicament, one tends to create, both in animals and in man, a strain or race which resists the arsenic chemotherapy to such an extent that the term arsenic-fastness has come into existence as a brief designation of the modified strain.[1] A similar phenomenon has been observed in spirochetosis in fowls and mammals. Even with a typical bacterium, there seems to exist a possibility of raising the original resistance against a certain arsenic compound to a considerable degree, as was shown by Marks[2] in his experiment with the paratyphoid bacillus, which he was finally able to cultivate in a medium containing eight times the amount of arsenious acid which the organism was able to withstand at the beginning of his experiment. Marks accomplished this within a period of 3 years by successive transplantations from a weaker to a gradually stronger concentration of the acid.

In syphilis, even before the discovery of *Treponema pallidum*, it had long been suspected that the causative agent of this disease acquires a gradual tolerance to the action of mercurial and iodide preparations. The intermittent form of treatment generally adopted by clinicians, with gradually ascending doses of the medicaments for each course, bears sufficient evidence of this assumption. That

[1] Ehrlich, P., and Hata, S., Die experimentelle Chemotherapie der Spirillosen, Berlin, 1910.

[2] Marks, L. H., Ueber einen arsenfesten Bakterienstamm, *Z. Immunitätsforsch., Orig.*, 1910, vi, 293.

unicellular organisms, under certain environmental influences, gradually acquire tolerance to certain toxic substances is not inconceivable, inasmuch as instances are not wanting where a group of cells, or an entire organization of much higher multicellular organisms, becomes adjusted to new surroundings when the change is brought about by degrees. In short, the state of drug-fastness is well known among the metazoan organisms.

To establish definitely whether *Treponema pallidum* acquires an increased resistance to various antisyphilitic therapeutic agents is of great practical importance, for the regulation of dosage of medicaments must necessarily be guided by the changes which occur at the same time in the resistance of the parasites. It may be recalled that Ehrlich assumed from analogy with trypanosomiasis and fowl spirochetosis that *Treponema pallidum* becomes readily fast or insensitive to the action of arsenic compounds, and with this idea he evolved his *therapia sterilisans magna*, that is, a total sterilization of *Treponema pallidum* in the system of the infected individual by a single administration of salvarsan, in which he combined a maximum parasitotropic and a minimum organotropic activity. The benefit derived from his efforts to achieve the aim of *therapia sterilisans magna* has been great, but in its practical application salvarsan has fulfilled only a part of Ehrlich's expectation. The consensus of opinion is that salvarsan has to be used in many successive doses, like other antisyphilitic medicaments, in order to obtain the full benefit of the drug. The introduction of salvarsan and neosalvarsan as routine medicaments in the treatment of syphilis raises once more the question whether or not the repeated injections of these preparations tend to produce an arsenic-fast strain of *Treponema pallidum*. This vital point, as well as those with regard to the alleged fastness of the *pallidum* against mercurial and iodide compounds, has never been proved experimentally, probably because of the fastidious character of *Treponema pallidum* in its behavior in the experimental animals available for studying these points.

For ascertaining experimentally that the syphilitic organism becomes gradually more tolerant to the sterilizing effect of various arsenic, mercurial, and iodide preparations, four different methods suggest themselves: (1) to determine *in vivo* the resistance of *Trep-*

onema pallidum to the germicidal action of the drug in question, both before and after the administration of the drug; (2) to subject *in vitro* the *pallidum* derived from normal and treated animals to the action of various concentrations of the drug and then to inoculate them into a series of suitable animals; (3) to cultivate the organism instead of inoculating it into animals as in (2); (4) to determine the degrees of tolerance of pure cultures of *Treponema pallidum* to a given medicament by testing the organisms *in vitro* against gradually increasing doses of the drug.

The first two methods are practically unavailable for the present problem, since there is no animal in which an accurate estimation of the relation between the number of the parasites and the severity and character of the lesions produced could be made with uniformity, and no method is yet known which will permit the cultivation of the *pallidum* directly from the animal tissue emulsions, as would be required if we were to adopt the third procedure. We have, therefore, left these three methods out of consideration and followed the fourth, the only procedure which may be carried out with the various factors under ready control and with a fair degree of constancy in results.

In the present work, we have chosen not only the syphilitic spirochete, but also some allied non-pathogenic varieties, and tested them against some of the representatives of antisyphilitic medicaments. We do not consider that the phenomena observed *in vitro* on pure cultures of spirochetes can be directly transferred to the processes occurring in the animal or human body, but we believe that the observations reported in this paper warrant sufficient interest as such.[3]

EXPERIMENTAL.

Method.

As the test objects, three strains of pure cultures of *Treponema pallidum*, one of *Treponema microdentium*, and one of *Spirochæta refringens* were employed. Against each of the above organisms, salvarsan, neosalvarsan, bichloride of mercury, and Lugol's solution

[3] Akatsu, S., The Resistance of Spirochetes to the Action of Hexamethylenetetramine Derivatives and Mercurial and Arsenic Compounds, *J. Exp. Med.*, 1917, xxv, 363.

were used. The solutions of salvarsan and neosalvarsan in sterile distilled water were each time freshly prepared, the proportion being 1 : 10,000. The solution of bichloride of mercury was made up to 1 : 10,000 during the early part of the experiment and became 1 : 1,000 as the concentration was gradually increased in order to keep the volume of the solution of the sublimate to be added to the media within a certain limit. Lugol's solution was prepared by dissolving 1 gm. of iodine and 2 gm. of potassium iodide in 300 cc. of distilled water. In the experiment a 1 : 10 dilution was first used, then an undiluted solution, (1 part of iodine, 2 parts of potassium iodide, and 300 parts of distilled water), and finally one three times as concentrated as the usual undiluted solution (1 part of iodine, 2 parts of potassium iodide, and 100 parts of distilled water).

Because of certain technical difficulties, the use of solid media was restricted to a small series. The majority of the experiments were carried out by means of a fluid medium consisting of equal parts of neutral bouillon and ascitic fluid with a piece of fresh kidney of a normal rabbit. To maintain the relative volumes of the drug solutions and the culture media, none of the former was added in a volume greater than 1 cc. or less than 0.05 cc. When the requisite amount of the drug solution exceeded 1 cc., a corresponding quantity of a tenfold stronger concentration was employed in order to keep the volume within the specified limit. The total volume of the culture medium in each tube was made uniformly 5 cc., including the quantity of drug solution added.

A series of test-tubes was set up, each containing a piece of fresh rabbit kidney, and inoculated with the pure culture of the spirochete. To the tubes were added first the ascitic bouillon (equal parts), and then the drug solution in the quantity indicated for each tube. The whole content of the tube was thoroughly mixed by gently inverting the tube a few times, then covered with a layer of sterile paraffin oil, and finally incubated at 36°C. in an anaerobic apparatus. At the end of 14 days, the cultures were examined for growth under the dark-field microscope. From the tubes showing varying degrees of growth, transfers were made into a new series of media containing different doses of the drug. Again, after a fortnight's incubation, transfers were similarly made, the process being repeated every 2 weeks.

Results of Experiments.

It was possible to find certain quantities of each drug, the addition of which to the culture media completely inhibited the growth of the organisms. As the quantities of the drug were gradually reduced, the organisms grew in corresponding abundance. With a very small quantity of the drug, no appreciable effect upon the growth was seen. But between the two extremes there were doses which distinctly showed the inhibitory influence of the drug as indicated by the retardation and meagerness of growth. In the border-line tubes the organisms were fewer in number, less active, and often granular in appearance. Numerous degenerated forms were seen in these tubes.

To conform with the purpose of the present work, transfers were made both from the border-line tubes and from those containing smaller amounts of the drug into a number of new media, to some of which ascending doses of the drug solution had been added. After 14 days in the incubator at 36°C., the cultures were examined for growth in the manner already described. From repeated observations it was discovered that no growth or very scant growth was obtainable when a transfer was made from one of the border-line tubes to a tube containing an equal quantity of the drug, and that even if there was any growth in the second transfer, it was not further transferable. The organisms seemed to succumb to the continued effect of the drug in such a concentration. Under these conditions there was no evidence that the organisms had in any way acquired an increased resistance to the drug.

On the other hand, the organisms growing fairly well in the tubes with somewhat smaller amounts of the drug were found capable of transplantation into new media, in the presence of not only the same amount, but also of somewhat larger doses. In other words, the minimal inhibitory doses became gradually larger with each transfer. In the accompanying tables we present a general aspect of the effect of drug-containing media upon the gradual development of tolerance by the spirochetes.

The point of interest here lies in the fact that the organisms under the influence of moderate quantities of an adverse drug acquire a greater resistance to it and are capable of doing so progressively up to a certain point. As will be described, the various organisms em-

ployed in this experiment behaved differently towards different agents, and our results permit the tentative generalization that the group of treponemata gains, under certain conditions, a definite, though rather slight degree of resistance to salvarsan and neosalvarsan, while its resistance to the bichloride of mercury (and iodide?) preparation is strikingly augmented under similar circumstances.

In Tables I and II the reaction of different spirochetes towards the influence of each of the chemical preparations used in the present work is shown.

TABLE I.

First transplantation from drug media to drug media.		Transferred to the tubes containing.				
		Dose.				No drug.
		1	2	3	4	
From tubes containing the doses and showing the growth indicated.	Dose 1, causing total inhibition (−).	−	−	−	−	−
	Dose 2, causing slight growth (<+).	−	−	−	−	+
	Dose 3, causing moderate growth (<+).	−	<+	+	+	+
	Dose 4, causing good growth (+).	−	+	+	+	++
	Dose 5, causing no inhibition (++).	−	<+	<+	+	++
	Control tube without any drug, showing luxuriant growth (++).	−	<+	<+	+	++

TABLE II.

Third transplantation from drug media to drug media.		Transferred to the tubes containing.				
		Dose.				No drug.
		1	2	3	4	
From the second transplants containing the doses and showing the growth indicated.	Dose 2, showing moderate growth (<+).	<+	+	++	++	++
	Dose 3, showing good growth (+).	<+	+	++	++	++
	Dose 4, showing good growth (+).	<+	+	++	+	++
	Control tube without any drug, showing luxuriant growth (++).	−	<+	<+	+	++

Table III represents the results of experiments with salvarsan and shows how various *pallidum* strains, and *microdentium* and *refringens*, withstood the action of the drug.

TABLE III.

Salvarsan.

Maximum Doses in Which Abundant Growth Still Occurred on Successive Transplantations.

	Generation in drug media.						
	1	2	3	4	5	6	7
T. pallidum.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Strain McD.....	0.03	0.05	0.06	0.07	0.07	0.08	0.1
" R.....	0.02	0.03	0.04	0.04	0.05	0.1	0.12
" Z. A.....	0.02	0.04	0.04	0.05	0.08	0.12	Accident
T. microdentium.....	0.01	0.02	0.04	0.06	0.07	0.07	"
S. refringens.....	0.02	0.02	0.04	Accident.	0.04	0.04	0.05

The figures denote the amount in milligrams of salvarsan contained in 5 cc. of the culture medium. The first column shows the doses of salvarsan in which various strains of the spirochetes still thrived; the second those in which the organisms grew well on the first transplantation; the third those for the second transplantation, etc. It will be seen that the initial tolerance of the *pallidum* and *refringens* was slightly greater than that of the *microdentium*, while the rate with which tolerance increased, up to the end of the fifth and sixth transplantation, was greatest with the *microdentium* and *pallidum*, these having attained about six to seven times their original tolerance within $3\frac{1}{2}$ months. The *refringens* still remained sensitive to the drug during this time.

Table IV shows the results with neosalvarsan, and they agree in general with those obtained with salvarsan, except that larger quantities were tolerated on account of its weaker action. The *pallidum* and *microdentium* reached four to five times their initial tolerance, while the tolerance of *refringens* was least affected.

In general, the tolerance of the *pallidum* and *microdentium* to salvarsan and neosalvarsan was definitely raised, while that of the *refringens* seems to have been least increased. With the *pallidum*

TABLE IV.

Neosalvarsan.

Maximum Doses in Which Abundant Growth Still Occurred on Successive Transplantations.

	Generation in drug media.						
	1	2	3	4	5	6	7
T. pallidum.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Strain McD	0.03	0.07	0.08	0.1	0.12	0.15	0.15
" R	0.02	0.05	0.06	0.06	0.07	0.08	0.08
" Z. A.	0.03	0.04	0.06	0.06	0.07	0.08	0.1
T. microdentium	0.02	?	0.03	0.07	0.1	0.1	0.1
S. refringens	0.03	0.06	0.08	0.08	0.1	0.1	0.1

strains, the rise in tolerance was gradual and the probable limit was not reached until the fifth or sixth transfer, which was made at the end of the 12th or 14th week. With the *microdentium* and *refringens*, especially the latter, the limit of tolerance was reached much sooner, and it could not be raised further after the fourth transfer. In salvarsanized media, the *refringens* reached the limit on the second transfer and remained unchanged for the rest of the experimental period.

The results obtained with bichloride of mercury, compared with those with salvarsan and neosalvarsan were striking. Table V shows that the initial tolerance of all the spirochetes was almost the same, being 0.01 mg. per 5 cc. of the medium. One of the *pallidum* strains tolerated 0.02 mg. The increase in tolerance rapidly rose from the first transfer and progressed gradually for the following generations. In *Treponema pallidum* the limit was not reached until the 16th week (seventh transfer), while that of the *microdentium* had already been reached on the 8th week (third transfer), and that of the *refringens* on the 12th week (fifth transfer). Even the *pallidum* strains could not tolerate much higher concentrations, the absolute limit being 1 mg. in 5 cc. of the medium, when the color of the tissue, as well as of the ascitic fluid, becomes gray within a few days at 36° C., and black streaks appear in the tissue.

The striking feature of the experiment is the high rate of increase in tolerance through the organisms' becoming accustomed to the

TABLE V.

Bichloride of Mercury.

Maximum Doses in Which Abundant Growth Still Occurred on Successive Transplantations.

	Generation in drug media.							
	1	2	3	4	5	6	7	8
T. pallidum.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Strain McD.	0.02	0.07	0.13	0.17	0.2	0.23	0.5	0.7
" R.	0.01	0.03	0.07	0.1	0.2	0.3	0.7	
" Z. A.	0.01	0.02	0.07	0.07	0.15	0.3	0.5	0.5
T. microdentium.	0.01	0.02	0.07	0.1	0.1	0.1	0.1	0.1
S. refringens.	0.01	0.015	0.03	0.07	0.1	0.3	0.3	0.3

mercurial salt. With the *pallidum*, it was between 35 and 70 times the initial tolerance; with the *refringens*, about 30 times; and with the *microdentium*, 10 times. We did not believe that these delicate organisms could be made resistant to the action of so simple and powerful an inorganic disinfectant as bichloride of mercury. The above observations seem to point to the possibility that bichloride of mercury forms, with certain constituents (proteins and lipoids) of the culture medium, a compound which induces tolerance on the part of the spirochetes.

In Table VI the results obtained with Lugol's solution are given. The figures in the first and second columns do not indicate the maximum doses in which the spirochetes could grow, but those which

TABLE VI.

Lugol's Solution.

Amounts Contained in Successive Transplantations.

	Generation in drug media.					
	1	2	3	4	5	6
T. pallidum.	cc.	cc.	cc.	cc.	cc.	cc.
Strain McD.	0.03	0.05	0.5	1.5	2.5	2.5
" R.	0.03	0.04	0.5	0.7	1.5	2.5
" Z. A.	0.02	0.05	0.5	1.0	2.0	2.0
T. microdentium.	0.01	0.06	0.3	0.8	0.8	2.0
S. refringens.	0.02	0.03	0.3	0.5	1.0	2.0

had been arbitrarily added to the media at the beginning of the experiments, with the purpose of gradually accustoming the organisms to the action of iodine and iodide. The doses given in the third to the sixth columns indicate the quantities added to the media where the spirochetes could still grow well. It may be stated here that these organisms, before passing through the iodinized media for some generations, could not grow in the presence of 0.7 cc. of Lugol's solution in 5 cc. of the culture media.

The color of Lugol's solution soon disappears from the media when the mixture is placed at 36°C. Even the addition of 2.5 cc. did not preserve the brown color over 48 hours. The most important factor affecting the antispirochetal action of the iodine solution was found to be the size of the fresh tissue added. The larger the tissue, the safer the spirochetes were from the inhibitory or sterilizing influence of the preparation. For this reason our experiments were carried out with as uniform a size of the tissue as practicable for each tube, and the results presented in Table VI may be considered approximately correct. It is true also with salvarsan, neosalvarsan, and bichloride of mercury, but the interference from this source was much less disturbing than with the iodine solution. Thus our figures in all the experiments should be taken as expressing merely the approximate values.

At the end of the experiments, a comparative study of the drug-fast and the ordinary stock cultures of the spirochetes in relation to their resistance to salvarsan, neosalvarsan, bichloride of mercury, and Lugol's solution was undertaken. The results are shown in Tables VII, VIII, IX, and X.

Attempts to carry out parallel experiments in solid media met with certain technical difficulties, in that the drugs had to be added while the mixture of the ascitic fluid and agar was still in fluid condition; that is, at a temperature of about 45°C. When salvarsan, neosalvarsan, and bichloride of mercury were mixed with the media, a distinct turbidity resulted, and solidification of the media at once followed, sometimes rendering a uniform distribution of the drug impossible. It was for this reason probably that we were unable to obtain results of definite constancy to warrant the assumption of the existence of an increased drug tolerance in the spirochetes when

TABLE VII.

Salvarsan.

In 5 cc. of media containing.	Strain McD., which grew in the tube containing 0.06 mg.	Strain McD., never in contact with salvarsan.
	Growth.	Growth.
mg.		
0.2	−	−
0.1	<+	−
0.05	+	−
0.01	++	+
0.005	++	++
No drug.	++	++

TABLE VIII.

Neosalvarsan.

In 5 cc. of media containing.	Strain R., which grew in the tube containing 0.1 mg.	Strain R., never in contact with neosalvarsan.
	Growth.	Growth.
mg.		
0.2	−	−
0.1	<+	−
0.05	<+	−
0.01	+	<+
0.005	++	<+
No drug.	++	++

TABLE IX.

Bichloride of Mercury.

In 5 cc. of media containing.	Strain Z. A., which grew in the tube containing 0.5 mg.	Strain Z. A., from original solid culture, never in mercuric chloride media.
	Growth.	Growth.
mg.		
1.0	−	−
0.5	<+	−
0.1	+	−
0.05	++	−
0.01	++	<+
No drug.	++	++

TABLE X.

Lugol's Solution.

In 5 cc. of media containing.	Strain McD., which grew in the tube containing 2 cc.	Strain McD., never in contact with Lugol's solution or iodide.
	Growth.	Growth.
cc.		
2.0	<+	−
1.0	+	−
0.5	++	±
0.1	++	+
0.05	++	+
No drug.	++	++

passed through the drug-containing media for several generations. Nor was it possible to demonstrate the acquired drug tolerance by means of solid media, which was easily seen in the fluid media. We are not prepared to offer an explanation for this phenomenon. Perhaps in fluid media the drugs enter into combination with certain tissue ingredients of the media and in this form induce a gradual tolerance on the part of the spirochetes, whereas with solid media the drugs are held in the agar in such a manner as not to become modified enough to produce increased resistance in the spirochetes.

With regard to the duration of the acquired drug tolerance of the spirochetes, a series of experiments was carried out in which the drugged strains were returned to the ordinary fluid media without any drug. From each successive generation, tolerance tests were made with varying concentrations. The results are summarized in Table XI.

As may be seen from Table XI, the acquired tolerance to various drugs decreases fairly rapidly. Within a period of 8 weeks, during which four transfers through ordinary media were made, the acquired tolerance of the *pallidum* and *refringens* to salvarsan and neosalvarsan entirely disappeared, and that of *microdentium* disappeared even sooner. The disappearance of the acquired tolerance to bichloride of mercury was more gradual, requiring about 10 weeks for complete restoration of the normal sensitiveness to this salt. In this respect, the paratyphoid strain used by Marks[2] differs greatly from the spirochetes, as the former passed through forty-six passages before returning to the initial tolerance.

TABLE XI.

Maximum Doses of Each Drug in Which Various Drug-Fast Strains of the Spirochetes Still Grew Well after Being Returned to Drug-Free Media for Successive Generations.

	Salvarsan.			Neosalvarsan.			Bichloride of mercury.		
	*T. pallidum.**	*T. microdentium.*	*S. refringens.*	*T. pallidum.**	*T. microdentium.*	*S. refringens.*	*T. pallidum.**	*T. microdentium.*	*S. refringens.*
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
After one generation in drug-free media....	0.1	0.05	0.05	0.15	0.1	0.1	0.5	0.1	0.1
" two generations in " "	0.05	0.03	0.03	0.1	0.05	0.08	0.3	0.1	0.1
" three " " " " ...	0.05	0.02	0.02	0.05	0.02	0.04	0.05	0.07	0.05
" four " " " "	0.03	0.01	0.02	0.03	0.02	0.03	0.04	0.04	0.03
" five " " " "	0.02	0.01	0.02	0.03	0.02	0.03	0.02	0.01	0.01
" six " " " "	0.02	0.01	0.02	0.03	0.02	0.03	0.02	0.01	0.01

* *Pallidum*, Strain McD.

SUMMARY.

In the foregoing experiments we attempted to determine whether or not, by subjecting several varieties of spirochetes to increasing doses of certain chemotherapeutic agents, a gradual increase of resistance to the latter could be shown. For this purpose, pure cultures of *Treponema pallidum*, *Treponema microdentium*, and *Spirochæta refringens* were used against the action of salvarsan, neosalvarsan, bichloride of mercury, and iodine-iodide potassium solution *in vitro*. For culture media, the usual ascites-broth-tissue medium as well as solid ascites-agar-tissue medium was used. After permitting the spirochetes to grow for a fortnight in media containing certain quantities of each drug, transfers were made from tubes showing various degrees of growth to the next series of tubes containing the same drug in still higher concentrations, and similar transfers repeated every 2 weeks. The results of the experiments may be briefly summarized as follows:

1. *Treponema pallidum* and *Treponema microdentium* have, within 3 to 4 months, increased their tolerance to salvarsan and neosalvarsan to five and one-half times their original mark. With *Spirochæta refringens* the increase was about three times.

2. Against the action of bichloride of mercury, the amount of increased tolerance of *Treponema pallidum* was about 35 to 70 times the original, while that of *Treponema microdentium* was about 10 times as much and was reached within 10 weeks. *Spirochæta refringens* resisted 30 times the original dose.

3. There was an unmistakable increase of resistance of these spirochetes to the action of the iodine-iodide solution (Lugol's solution) when they were grown for several generations in fluid media containing the iodine solution, but the rate of increase between the initial and the acquired tolerance was slight. In general, the addition of Lugol's solution to fluid media has a weak inhibitory influence upon the growth of the spirochetes, requiring for the total suppression of growth a quantity of over 0.7 cc. to 5 cc. of the culture media. The tolerance reached was for about three times that amount.

4. A similar tolerance phenomenon has not been established when employing a solid instead of a fluid medium containing the drugs. No explanation is offered except a suggestion that the drugs held in the agar do not enter into combination with certain tissue constituents of the medium as they are able to do with tissue elements in fluid media. This may be a factor necessary for inducing drug tolerance in these organisms *in vitro*.

5. The increased drug-fastness *in vitro* has a limit beyond which no further advance can be made. This limit varies with different species of spirochetes.

6. The acquired drug-fastness *in vitro* gradually disappears when the spirochetes are cultivated again in the drug-free media for several generations.

THE RESISTANCE OF SPIROCHETES TO THE ACTION OF HEXAMETHYLENETETRAMINE DERIVATIVES AND MERCURIAL AND ARSENIC COMPOUNDS.

By SEINAI AKATSU, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, November 22, 1916.)

The effect of the most common physical and chemical agents upon pure cultures of different spirochetes has already been studied by Bronfenbrenner and Noguchi.[1] In the present series of experiments we studied the action of a large number of hexamethylenetetramine derivatives and new mercurial and arsenic compounds upon several varieties of spirochetes in pure cultures. The bactericidal strengths of the drugs upon different bacilli or cocci have already been tested,[2–5] and it seemed to us to be of sufficient interest to examine also their germicidal powers for spirochetes, since spirochetes and bacteria often show a marked difference with regard to their resistance to a given chemical substance.

[1] Bronfenbrenner, J., and Noguchi, H., On the Resistance of Various Spirochaetes in Cultures to the Action of Chemical and Physical Agents, *J. Pharm. and Exp. Therap.*, 1912–13, iv, 333.

[2] Jacobs, W. A., and Heidelberger, M., The Quarternary Salts of Hexamethylenetetramine. I–VIII, *J. Biol. Chem.*, 1915, xx, 659, 685; xxi, 103, 145, 403, 439, 455, 465; Mercury Derivatives of Aromatic Amines. I. Contribution to the Structure of Primary and Secondary *p*-Aminophenylmercuric Compounds, xx, 513.

[3] Jacobs, W. A., The Bactericidal Properties of the Quarternary Salts of Hexamethylenetetramine. I. The Problem of the Chemotherapy of Experimental Bacterial Infections, *J. Exp. Med.*, 1916, xxiii, 563.

[4] Jacobs, W. A., Heidelberger, M., and Amoss, H. L., The Bactericidal Properties of the Quarternary Salts of Hexamethylenetetramine. II. The Relation between Constitution and Bactericidal Action in the Substituted Benzylhexamethylenetetraminium Salts, *J. Exp. Med.*, 1916, xxiii, 569.

[5] Jacobs, W. A., Heidelberger, M., and Bull, C. G., The Bactericidal Properties of the Quarternary Salts of Hexamethylenetetramine. III. The Relation between Constitution and Bactericidal Action in the Quarternary Salts Obtained from Halogenacetyl Compounds, *J. Exp. Med.*, 1916, xxiii, 577.

EXPERIMENTAL.

The new compounds employed for the present work[6] are given in Table I. Most of these compounds are readily soluble in distilled water. A few exceptions are marked with an asterisk or a dagger in the table. The insoluble preparations were first ground in a mortar with a small quantity of water and then 0.1 N hydrochloric acid (to those marked with an asterisk) or 0.1 N sodium hydroxide (to those marked with a dagger) was added, until the drug passed into a clear solution. After that more water was added until a 1 per cent solution was obtained. Most of these water-insoluble drugs required from 2 to 4 cc. of a 0.1 N solution of hydrochloric acid or sodium hydroxide to dissolve 0.1 gm. of the substance.

To determine the intensity of the action of these chemical compounds, the following procedure was adopted. A number of dilutions in descending strengths were made with each compound, such as 0.01, 0.001, 0.0001, etc., using distilled water as a diluent. From each of the dilutions 1 cc. was measured into each of the small test-tubes in series, and to the latter three drops of a stock culture of the spirochetes were added.

The stock culture used in the first series of experiments consisted of fluid cultures of *Treponema pallidum* as grown by Noguchi's method[7] and contained a considerable number of actively motile organisms, as seen under the dark-field microscope.

The contents of each tube having been thoroughly mixed by shaking, the tubes were left in a water bath and kept at 37°C. for 1 hour. At the end of that time, transplants into new media were made with the contents of the tubes, in order to determine in what dilution each of the chemical compounds was able to sterilize the spirochetes completely. A microscopic examination alone is insufficient to determine whether the organisms are killed or not. For the purpose of setting up new cultures, Noguchi's solid medium containing

[6] I wish to acknowledge the courtesy of Dr. W. Jacobs and Dr. M. Heidelberger, of The Rockefeller Institute for Medical Research, who kindly placed these chemical compounds at my disposal.

[7] Noguchi, H., A Method for Cultivating *Treponema pallidum* in Fluid Media, *J. Exp. Med.*, 1912, xvi, 211.

TABLE I.

Preparation No.	
9	*p*-Bromobenzylhexamethylenetetraminium chloride.
16	*o*-Xylylenedihexamethylenetetraminium "
19	2-Nitro-3, 4-dimethoxybenzylhexamethylenetetraminium chloride.
21	2-Oxy-3-carbomethoxynaphthobenzylhexamethylenetetraminium chloride.
28	5-Chloromethylvanillin + hexamethylenetetramine.
29	5-Chloromethylsalicylic acid + "
40	*p*-Iodobenzylhexamethylenetetraminium bromide.
46	*o*-Nitrobenzylhexamethylenetetraminium chloride.
47	*p*-Nitrobenzylhexamethylenetetraminium "
50	Methylhexamethylenetetraminium iodide.
84	Chloroacetamide + hexamethylenetetramine.
86	Oxymethylchloroacetamide + "
90a	Ethyl bromoacetate + "
96	Chloroacetylaniline + "
97	β-Acetoxy-α-chloroacetylnaphthobenzylamine + hexamethylenetetramine.
102	Chloroacetyl-α-naphthylamine + hexamethylenetetramine.
107	Chloroacetylbenzylamine + hexamethylenetetramine.
109	Chloroacetyl-β-naphthylamine + "
111	*o*-Methylchloroacetylbenzylamine + "
112	*p*-Chloroacetylaminobenzoic ethyl ester + hexamethylenetetramine.
114	Chloroacetylurea + hexamethylenetetramine.
121	Phenoxyethylhexamethylenetetraminium bromide.
122	*p*-Bromochloroacetylaniline + hexamethylenetetramine.
126	Chloroacetylaminoazotoluene + "
134	Chloroacetyl-*p*-anisidine + "
138	Chloroacetylacetylphenylhydrazine + hexamethylenetetramine.
142	Chloroacetethylamide + hexamethylenetetramine.
146	Menthyl bromoacetate + "
147	Bromoethylphthalimide + "
148	*p*-Nitrobenzoic bromoethyl ester + hexamethylenetetramine.
150	Bromoethyl benzoate + hexamethylenetetramine.
158	β-Iodopropionyl-*o*-anisidine + "
163	*p*-Ethoxyphenyl bromoethyl ketone + hexamethylenetetramine.
164	Chloroacetyl-ψ-cumidine + hexamethylenetetramine.
168	*p*-Acetamino-ω-bromoacetophenone + "
171	*m*-Chloroacetylaminomethyl benzamide + "
172	*m*-Chloroacetyl-α, α-phenylbenzylhydrazine + hexamethylenetetramine.
174	*m*-Chloroacetylaminoethyl anisate + hexamethylenetetramine.
204	3-ω-Bromoacetylquinaldine + "
218	Tribromo-*p*-cresyl bromoethyl ether + "
219	*p*-Chloroacetylaminoleucomalachite green + " "
229	*p*-Chloroacetylaminobenzeneazo-*p'*-dimethylaniline + hexamethylenetetramine.
232	*p*-Chloroacetylaminobenzeneazo-*p'*-diethylaniline + "

TABLE I—*Concluded.*

Preparation No.	
234	α-Naphthyl bromoethyl ether + hexamethylenetetramine.
239	*o*-Acetaminophenyl bromoethyl ether + "
242	*p*-Chloroacetylaminodiethylaniline + "
244	Chloroacetylaminoethyl *p*-nitrobenzoate + "
249	*p*-Chloroacetylaminodipropylaniline + " *
252	*p*-Chloroacetylaminotetraethyl-*p'*, *p''*-diaminotriphenylmethane + hexamethylenetetramine.
253	Chloroacetyldiethylamine + hexamethylenetetramine.
255	*p*-Cyanobenzylhexamethylenetetraminium chloride.
257	*o*-Chloroacetylaminophenyl benzoate + hexamethylenetetramine.
261	Chloroacetyltriphenylmethylamine + "
262	Chloroacetylleucoauramine + " *
263	Chloroacetylaminoethyl *o*-nitrobenzoate + "
267	Chloroacetylaminoethyl β-naphthoate + "
271	Chloroacetyl-N-phenylaminoethyl *p*-nitrobenzoate + hexamethylenetetramine.
272	*m*-Acetamino-*p*-tolyl ω-iodoethyl ketone + "
273	Chloroacetylethylaminoethyl *p*-nitrobenzoate + "
278	α, β-Diphenylchloroacetylaminoethanol + "
280	*m*-Chloroacetylaminoacetophenone + "
282	α-Phenyl-α-oxy-β-chloroacetylaminoethane + hexamethylenetetramine.
283	*p*-Nitrobenzoylaminoisopropyl chloroacetate + "
286	Iodopropanol + hexamethylenetetramine.
289	2-Chloroacetylamino-3-oxy-3-methylbutane + hexamethylenetetramine.
291	Chloroacetyl-*o*-methylphenoxyethylamine + "
293	β-Chloroacetylamino-γ-butanol + "
298	β-Phenyl-β-oxy-γ-chloroacetylaminopropane + "
301	β-Naphthyl bromoethyl ether + hexamethylenetetramine.
303	2-Oxy-3, 5-dibromobenzyl bromide + " † ‡
308	Chloroacetyl-*m*-iodoaniline + "
309	Chloroacetyl-5-iodo-*o*-toluidine + "
M1	4-[*p*-Oxybenzeneazo]-phenylmercuric acetate.
M4	[*o*-Oxybenzylideneamino]-phenylmercuric " †
M7	1-Amino-2-[*p*-naphthaleneazophenylmercuric acetate]-5-sulfonic acid.

* Grind up in a mortar with a little water and add 0.1 N hydrochloric acid until dissolved.

† Treat as above, using 0.1 N sodium hydroxide instead of hydrochloric acid.

‡ Difficultly soluble.

a piece of fresh sterile rabbit kidney and ascitic agar (17 cc. in each tube) was employed. The quantity of transplanted fluid containing the spirochetes and the drug was approximately 0.2 cc. for each

tube. Thus, a certain amount of the drug was necessarily carried over into a new culture together with the spirochetes, which were suspended in different solutions of the drug. After 14 days' incubation at 37°C., the results of the culture were ascertained by both macroscopic and dark-field examinations.

In the preliminary series of experiments it was found that different drugs varied considerably with regard to their spirocheticidal power. Thus, some had the power to kill the organism in a dilution of 1:100, but not in 1:1,000, while others were effective in 1:1,000 and not in 1:10,000, etc. Each of the drugs was again subdivided for the purpose of determining more accurately its disinfecting power against the spirochetes. For example, in the case of a drug which was found effective in 1:100 and not in 1:1,000, five subdivided dilutions between both extremes, such as 1:100, 1:250, 1:500, 1:750, 1:1,000, were tested.

In this series the manner of testing the spirocheticidal powers was exactly the same as in the first series. After 14 days the results were taken with the aid of the dark-field microscope (Table II). The color of each preparation, as it appears in a fresh 1 per cent solution, is noted in the table.

TABLE II.

Preparation No.	Appearance of a 1 per cent fresh solution.	Result.	
		Concentration sufficient to kill spirochetes.	Concentration in which spirochetes survived.
9	Clear.	1 : 1,000	1 : 2,500*
16	Brown.	1 : 2,500	1 : 5,000
19	Opaque.	1 : 2,500	1 : 5,000
21	Slightly greenish opaque with sediment.	1 : 2,500	1 : 5,000
28	Brown.	1 : 750	1 : 1,000
29	Clear.	1 : 2,500	1 : 5,000
40	Slightly opaque.	1 : 750	1 : 1,000
46	Opaque.	1 : 250	1 : 500
47	Clear.	1 : 750	1 : 1,000
50	"	1 : 100	1 : 250
84	"	1 : 1,000	1 : 2,500
86	"	1 : 250	1 : 500

* Grind up in a mortar with a little water and add 0.1 N sodium hydroxide until dissolved.

TABLE II—*Continued.*

Preparation No.	Appearance of a 1 per cent fresh solution.	Result.	
		Concentration sufficient to kill spirochetes.	Concentration in which spirochetes survived.
90a	Clear.	1 : 1,000	1 : 2,500
96	Slightly opaque.	1 : 1,000	1 : 2,500
97	" yellowish opaque.	1 : 1,000	1 : 2,500
102	Opaque.	1 : 500	1 : 750
107	Clear.	1 : 500	1 : 750
109	Milky opaque.	1 : 1,000	1 : 2,500
111	Slightly "	1 : 2,500	1 : 5,000
112	Opaque.	1 : 1,000	1 : 2,500
114	Clear.	1 : 1,000	1 : 2,500
121	"	1 : 250	1 : 500
122	Opaque.	1 : 2,500	1 : 5,000
126	Reddish yellow.	1 : 250	1 : 500
134	Slightly opaque.	1 : 2,500	1 : 5,000
138	Clear.	1 : 750	1 : 1,000
142	"	1 : 1,000	1 : 2,500
146	Slightly opaque.	1 : 750	1 : 1,000
147	Clear.	1 : 1,000	1 : 2,500
148	Slightly opaque.	1 : 250	1 : 500
150	Clear.	1 : 500	1 : 750
158	Milky opaque.	1 : 1,000	1 : 5,000
163	" "	1 : 500	1 : 750
164	" "	1 : 2,500	1 : 5,000
168	Clear.	1 : 750	1 : 1,000
171	" slight sediment.	1 : 1,000	1 : 2,500
172	Milky opaque.	1 : 2,500	1 : 5,000
174	Opaque.	1 : 500	1 : 750
204	Slightly reddish yellow.	1 : 2,500	1 : 5,000
218	Milky opaque.	1 : 2,500	1 : 5,000
219	Green.	1 : 5,000	1 : 7,500
229	Yellowish red.	1 : 500	1 : 750
232	Reddish yellow.	1 : 1,000	1 : 2,500
234	Clear.	1 : 500	1 : 750
239	"	1 : 1,000	1 : 2,500
242	Yellowish opaque.	1 : 1,000	1 : 2,500
244	Opaque.	1 : 2,500	1 : 5,000
249	Milky.	1 : 500	1 : 750
252	Green.	1 : 1,000	1 : 2,500
253	Clear.	1 : 1,000	1 : 2,500
255	"	1 : 1,000	1 : 2,500
257	Yellowish opaque, a little sediment.	1 : 1,000	1 : 2,500
261	Milky.	1 : 1,000	1 : 2,500

TABLE II—*Concluded.*

Preparation No.	Appearance of a 1 per cent fresh solution.	Result.	
		Concentration sufficient to kill spirochetes.	Concentration in which spirochetes survived.
262	Slightly opaque.	1 : 1,000	1 : 2,500
263	" yellowish opaque.	1 : 1,000	1 : 2,500
267	Milky.	1 : 2,500	1 : 5,000
271	Brown.	1 : 1,000	1 : 2,500
272	Milky with sediment.	1 : 5,000	1 : 7,500
273	Slightly opaque.	1 : 1,000	1 : 2,500
278	" " with sediment.	1 : 250	1 : 500
280	Clear.	1 : 1,000	1 : 2,500
282	"	1 : 1,000	1 : 2,500
283	Opaque.	1 : 500	1 : 750
286	Clear.	1 : 500	1 : 750
289	"	1 : 2,500	1 : 5,000
291	Opaque.	1 : 1,000	1 : 2,500
293	"	1 : 250	1 : 500
298	Clear.	1 : 1,000	1 : 2,500
301	Opaque.	1 : 1,000	1 : 2,500
303	Brown with slight sediment.	1 : 1,000	1 : 2,500*
308	Milky.	1 : 750	1 : 1,000
309	"	1 : 750	1 : 1,000
Atoxyl.	Clear.	1 : 50	1 : 100
M1	Yellow.	1 : 50,000	1 : 75,000
M4	Light yellow.	1 : 50,000	1 : 75,000
M7	Dark red.	1 : 25,000	1 : 50,000

A parallel series of experiments was conducted with a number of the most common disinfectants and antisyphilitic medicaments, in order to compare the action of new chemical compounds with that of standard ones. The results obtained with these substances are given in Table III.

These experiments were made with a strain belonging to a medium type of *Treponema pallidum*, as has been stated. For the comparison, two more strains, belonging one to a thin and the other to a thick type of the *pallidum*, as well as several other species of spirochetes (*refringens*, *microdentium*, *mucosum*, *calligyrum*, etc.) were tested with some of the chemicals. For this purpose M1, one of the powerful compounds against the *pallidum*, and No. 253, a much weaker drug, were chosen. The results are given in Table IV.

TABLE III.

Name of drug.	Appearance of a 1 per cent fresh solution.	Result.	
		Concentration sufficient to kill spirochetes.	Concentration in which spirochetes survived.
Phenol.	Clear.	1 : 2,500	1 : 5,000
Formalin.	"	1 : 750	1 : 1,000
Lysol.	Slightly opaque.	1 : 5,000	1 : 7,500
Bichloride of mercury.	Clear.	1 : 100,000	1 : 500,000
Salvarsan.	Yellow.	1 : 7,500	1 : 10,000
Neosalvarsan.	"	1 : 2,500	1 : 5,000
Sodium iodide.	Clear.	1 : 10	1 : 25
Potassium iodide.	"	1 : 10	1 : 25
Lugol's solution.	Deep reddish brown.	1 : 75	1 : 100
Iodoxybenzoic acid.	Clear.	1 : 500	1 : 1,000
Trypozofrol.	Dark red.	1 : 25,000	1 : 50,000
Neotrypozofrol.	Deep "	1 : 250	1 : 1,000
Sodium cholate.	" brown.	1 : 5,000	1 : 7,500
" glycocholate.	" "	1 : 2,500	1 : 5,000
" taurocholate.	Light "	1 : 2,500	1 : 5,000
" oleate.	Yellowish opaque.	1 : 7,000	1 : 10,000
Saponin.	" transparent.	1 : 7,500	1 : 10,000
Cholesterol.	Insoluble.	No action.	No action.
Cobra lecithide.	Yellowish opaque.	1 : 1,000	1 : 5,000
" venom.	" "	1 : 1,000	1 : 5,000

TABLE IV.

Spirochete.	M1			No. 253	
T. pallidum, large type.....	1:10,000	1:25,000	1:50,000	1:1,000	1:2,500
" " small "	−	+		−	+
" *calligyrum*.....		−	+	−	+
" *mucosum*.....		−	+	−	+
" *microdentium*.....		−	+	−	+
S. refringens.....		−	+	−	+

Different species of spirochetes seem to have about the same resistance to the action of these drugs.

In order to determine the relative resistance of spirochetes on the one hand, and certain bacteria on the other, *Bacillus dysenteriæ* Shiga and *Streptococcus* were subjected to the action of these compounds in a parallel series and under similar experimental con-

ditions. For this purpose, a 24 hour bouillon culture of the dysentery bacilli and an agar slant culture of streptococci were employed. The results are recorded in Table V.

TABLE V.

Preparation No.	*B. dysenteriæ.*				*Streptococcus.*		
	1:100	1:1,000	1:10,000	1:100,000	1:100	1:1,000	1:10,000
9	−	+					
16	+						
21	+						
29	+						
40	−	+			−	+	
46		−	+		−	+	
50	−	+			+		
84	−	+			−	−	+
86		−	+				
90a	−	+			−	+	
107	−	+			−	+	
114					−	+	
142	−	+			−	+	
146	−	+			−	+	
150	−	+			+		
171	−	+					
218	−	+					
232	−	+			−	+	
239	−	+					
242	+				−	+	
244	−	+			−	+	
253	−	+			−	+	
257		−	+				
278	−	+					
283	+				−	+	
286	−	+					
298	−	−	+				
Atoxyl.	+						
Lugol's solution.	−	+					
Neosalvarsan.	−	+		+			
Bichloride of mercury.	−	−	−	−			

DISCUSSION AND SUMMARY.

The majority of the 76 new compounds possessed spirocheticidal powers ranging between 1:1,000 (30) to 1:2,500 (14), while only 2 attained the power of 1:5,000, 1 of 1:25,000, and 2 of 1:50,000. On the other hand, 8 killed the spirochetes in a dilution of 1:750, 10 in 1:500, 7 in 1:250, 1 in 1:100, and 1 in 1:50.

It may be mentioned that the 2 (M1, M4) of 1:50,000 and 1 (M7) of 1:25,000 belong to the mercury compounds, and that mercuric chloride kills the organisms in a dilution of 1:100,000 under the same experimental conditions. It is also interesting to compare some of the more common chemicals and therapeutic reagents. Phenol is spirocheticidal in a dilution of 1:2,500, lysol in 1:5,000, formalin in 1:750, salvarsan in 1:7,500, and neosalvarsan in 1:2,500. Thus, of the new compounds there are at least 14 which have the same spirocheticidal power *in vitro* as has neosalvarsan.

It is of interest to note that nine compounds possessing the 1:1,000 spirocheticidal power showed only one-tenth of the antiseptic action when tested upon *Bacillus dysenteriæ* and *Streptococcus*, while some showed an even greater difference in this respect. Nos. 16, 21, 29, 218, and 244 were effective in a dilution of 1:2,500 for spirochetes and in a 1:100 or lower dilution for the bacteria just referred to. Preparations 46 and 84 appear to exert about the same effect both on the spirochetes and the bacteria, neither being very strong. Atoxyl killed the spirochetes in a 1:50 dilution.

One of the most striking results was obtained with various hemolytic substances. Neufeld and von Prowazek[8] found that spirochetes, unlike bacteria in general, are highly susceptible to the lytic action of sodium taurocholate and saponin, and they considered that this phenomenon was of differential diagnostic value in determining plant and animal organisms. Their observations were confirmed by Gonder,[9] who found, however, that spirochetes, especially treponemata,

[8] Neufeld, F., and von Prowazek, S., Ueber die Immunitätserscheinungen bei der Spirochaetenseptikämie der Hühner und über die Frage der Zugehörigkeit der Spirochaeten zu den Protozoen, *Arb. k. Gsndhtsamte.*, 1907, xxv, 494.

[9] Gonder, R., Spironemacea (Spirochaeten), in von Prowazek, S., Handbuch der pathogenen Protozoen, Leipsic, 6 Liefg., 1914, 671.

offer a great deal of resistance at the beginning, but finally undergo lysis, with their cell bodies swollen up or macerated. In the present experiment we have found that these substances not only bring about cytolysis of the spirochetes in higher concentrations, but also kill them without causing a gross destruction of the cells in very high dilutions; thus, sodium cholate in 1: 5,000, sodium glycocholate and taurocholate in 1: 2,500 each, saponin in 1:7,500. Sodium oleate, one of the most powerful hemolytic agents[10, 11] killed the spirochetes in 1: 7,000, and cobra lecithide and native cobra venom in a dilution of 1: 1,000 each.

Neither sodium nor potassium iodide displayed any marked spirocheticidal property (1: 10 only), while iodine in the form of Lugol's solution was able to kill them at 1: 75.

In conclusion I wish to acknowledge my indebtedness to Dr. Hideyo Noguchi for his assistance and advice.

[10] Madsen, T., and Noguchi, H., Toxines et antitoxines. Saponine-cholestérine, *Overs. k. Danske Vidensk. Selsk. Forh.*, 1904, 457.

[11] Noguchi, On Certain Thermostabile Venom Activators, *J. Exp. Med.*, 1906, viii, 87.

THE INFLUENCE OF CARBOHYDRATES ON THE CULTIVATION OF SPIROCHETES.

By SEINAI AKATSU, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

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While a newly isolated bacterium would usually, as a matter of bionomic routine, be tested soon for its power of fermentation of various carbohydrates, nothing systematic has been done in this direction with the group of organisms classed as spirochetes. The lack of investigation along this line may be partly due to the technical difficulties which still surround the cultivation of these organisms, and probably also to the limited number of workers in the field.

The present paper is a report of experiments which deal with the influence of starch and sugars upon pure cultures of various spirochetes. Attention has been directed to the fermentation phenomena as well as to the effect upon the growth and morphology of the organisms, since we are mindful of the fact that the addition of certain of these substances to some bacterial cultures may bring about almost incredible involution of forms[1] or sometimes induce spore formation in a bacterium which is otherwise not sporiferous.[1,2,3]

EXPERIMENTAL.

Method.

For the present work, the following substances were tested: amygdalin, arabinose, beerwort, dextrin, galactose, glycogen, glucose, inulin, lactose, levulose, maltose, mannite, raffinose, saccharose, and

[1] For example, Noguchi noticed that the colon bacillus undergoes striking morphological changes when cultivated in beerwort, producing gigantic pleomorphic forms of various shapes. Some of the large specimens measured about 30 μ in length and 2 or 3 μ in width in the swollen portion.

[2] Noguchi, H., Sporulation of the Group of *Bacillus aerogenes capsulatus*, *Proc. N. Y. Path. Soc.*, 1907, vii, 196.

[3] Fitzgerald, M. P., The Induction of Sporulation in the Bacilli Belonging to the *Aerogenes capsulatus* Group, *J. Path. and Bacteriol.*, 1911, xv, 147.

starch. These fifteen carbohydrates were added to the tubes containing the usual medium (equal parts of ascitic fluid and bouillon with a piece of fresh tissue, a total volume of 10 cc. to each tube) in a proportion of 1 : 100. Inoculations were then made into the media with each of the seven strains of *Treponema pallidum* and one each of *Treponema calligyrum*, *Treponema microdentium*, *Treponema mucosum*, and *Spirochæta refringens*. The culture tubes were covered with a layer of sterile paraffin oil and placed in an incubator at 36°C. Examinations of the spirochetes were made from time to time, for a period of 3 months with the first generation and of 2 months with the second generation.

Results of Experiments.

It was soon found that none of the spirochetes employed for the present study produced gas in the presence of these carbohydrates, and even after many weeks there was no turbidity or precipitate in any of the cultures which would indicate that the acid produced, if any, was sufficient in amount to cause coagulation of the proteins of the media. No difference in appearance could be discerned between the cultures in the sugar media and those in the sugar-free media, save the slight opalescence in the cultures of the *microdentium* in the glycogen and glucose media. When examined under the dark-field microscope, all the spirochetes were found to have grown vigorously in all the sugar media except those containing amygdalin, glycogen, glucose, and lactose. In the glycogen medium, the *microdentium* grew for several days, but quickly degenerated, though it multiplied abundantly in the beerwort medium. Glucose medium induced good growth of all the spirochetes in the first generation, but *microdentium* and some *pallidum* strains failed to grow when transferred to a new medium containing the same sugar. The effects of glycogen and glucose are similar. *Treponema mucosum*, which is similar to *Treponema microdentium* in morphology and odor-producing property, seems to be distinguished from the latter by its indifference to the addition of glucose to the medium. In Table I the results of the dark-field examination of the cultures are given as recorded after 3 weeks for the first generation and 4 weeks for the second.

The morphological features of the spirochetes were not appreciably influenced, except that the terminal appendages[4] were much more in evidence in the specimens grown in a sugar medium than in the sugar-free control cultures. In the culture of *microdentium*, the spirochetes showed numerous refractile spherical bodies, laterally attached, similar to those first described by Noguchi in the specimens of the *pallidum* cultures. Since the life of *Treponema microdentium* in sugar media is shorter than in a sugar-free medium, that is, since it grows earlier and degenerates earlier, the observed phenomenon may have an intimate connection with a process of degeneration such as plasmoptysis, where the minute granules do not represent a resistant form (spore) of the spirochetes, for they succumb to the degeneration as do the spirochetes themselves.

As may be seen from Table I, the addition of glycogen and glucose had a decidedly unfavorable effect upon the cultures of *Treponema calligyrum*, *Treponema microdentium*, *Treponema mucosum*, and some strains of the *pallidum*. *Treponema mucosum* grew most luxuriantly in all media except in that containing glycogen, and its uniform length, regular curves, and energetic motility indicate that this organism finds an ideal medium when these substances are added.

The reactions of the cultures varied from almost neutral to distinctly acid when tested with litmus paper. The acidity was strongest in the *microdentium* culture in glycogen and glucose media, and somewhat weaker in the media containing galactose, lactose, maltose, and inulin. Only a faint acidity was found in any of the cultures of spirochetes in sugar media except in glycogen and glucose media, where some of them produced a distinct acidity. In the control cultures without any carbohydrate, the reaction of the *microdentium* only was faintly acid, all the rest being practically neutral. By titrating the total acidity of 10 cc. of the fluid culture, it was found that the highest acidity as represented in the glycogen and glucose cultures of the *microdentium* was 0.1 N 4.8 cc., while in the cultures showing a weaker acidity, it was between 0.1 N 2 cc. to 0.1 N 3.2 cc. The ciphers for the control cultures varied from 0.1 N 0.8 cc. to 0.1 N 2 cc. Leaving a more exact qualitative and quantitative determination

[4] Noguchi, A Method for the Pure Cultivation of Pathogenic *Treponema pallidum* (*Spirochæta pallida*), *J. Exp. Med.*, 1911, xiv, 99.

TABLE I.

	Generation.	*T. pallidum.*							*T. calligyrum.*	*S. refringens.*	*T. microdentium.*	*T. mucosum.*
		McD.	No. 11	R.	C_2.	B29	B30	Z. A.				
Amygdalin.	1st.	+++	+	++	+++	+++	++	+++	++	+++	++	++
	2nd.	++	−	++	++	++	+	+	++	++	−	+
Arabinose.	1st.	+++	+++	+++	+++	+++	+++	+++	+++	+++	++g	+++
	2nd.	++	+++	+++	+++	++++	+++	+++	+++	+++	++	+++
Beerwort.	1st.	+++	+++	+++	+++	+++c	+++	+++	+++c	+++c	+++g	+++
	2nd.	+++	+++	+++	+++	+++	+++	+++	++	+++	++g	++
Dextrin.	1st.	+++	++	++	+++	+++	+++	++	+++	+++	+++	+++
	2nd.	+g	++	+	++	++	+++c	+++	++	+c	+g	+++
Galactose.	1st.	+	++	+++	+++	++	+++	+++c	++	++	+g	++
	2nd.	+++	+++	+++	+++	++	++	++	++	++	++	+++
Glycogen.	1st.	+++c	−	+++	++	++	++	++	−	+++	−	+
	2nd.	+	−	++	+	−	++	++	+	+	−	−
Glucose.	1st.	++c	+++	++	++	+++c	+++	+++c	+++c	+++c	++g	+++
	2nd.	++	−	+	+	++	+	−	+	++	−	++
Inulin.	1st.	+++	+++	+++	+++	+++c	+++	+++c	++	+++	++g	+++
	2nd.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+g	+++
Lactose.	1st.	++	++	++	++	+	++c	+++	++	+++c	++g	+++
	2nd.	+++	+++	+++	+++	+++	+++c	+++	+++	+++	+g	+++
Levulose.	1st.	+++	+++	+++	+++	+++c	+++	+++c	+++	+++	+++	+++
	2nd.	+++	+++	+++	+++	++++	+++	+++	++	++	++g	+++
Maltose.	1st.	+++c	+++	+++	+++	+++c	+++	++	+++	+++	+++	+++
	2nd.	+++	+++	+++	+++	+++	+++	+++	++	+++	+	+++
Mannite.	1st.	+++	+++	+++	+++	+++c	+++	+++c	+++	+++c	++g	+++
	2nd.	+++	+++	+++	+++c	+++c	+++	+++	+++	+++	++g	+++
Raffinose.	1st.	++c	++	++	+++	+++c	+++	+++c	++	+++	++g	+++
	2nd.	++c	+++	+++	+++	+++	++	++++	+++	+++	+++	+++
Saccharose.	1st.	+++	+++	++	+++c	+++	+++	+++	+++	+++	+++g	+++
	2nd.	++++	+++	++	+++	+++	+++	+++	+++	+++	++	+++
Starch.	1st.	+++	+++	+++	+++	+++	+++	++++	+++	+++	++g	++++
	2nd.	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++

In the table the sign − indicates absence of spirochetes; +, less than 100; ++, a number between 100 and 200; +++, more than 200; and ++++, innumerable spirochetes per field under Leitz oc. 3, $\frac{1}{12}$ oil immersion. The letter *c* denotes the presence of long threads or chains of spirochetes, while *g* indicates that there were numerous granular particles due to the degenerated spirochetes.

for another occasion, we believe that the fact has been established that some sugars are attacked to a certain extent by some of the spirochetes employed for the present experiment, and especially by *Treponema microdentium*. Of course, it is impossible from the present experiment to determine how much of the acidity found should be ascribed to the split products of the sugars. While the amount of acidity in the glycogen or glucose media did not produce coagulation of the proteins, nevertheless the early disintegration of the organisms, particularly in the case of *Treponema microdentium* shows the effect of the changes associated with the acid production in the media.

SUMMARY.

Various carbohydrates have been added to the fluid cultures of different strains of spirochetes in order to determine the behavior of the latter toward the carbohydrates. In the present experiment, amygdalin, arabinose, beerwort, dextrin, galactose, glycogen, glucose, inulin, lactose, levulose, maltose, mannite, raffinose, saccharose, and starch were tested with seven strains of *Treponema pallidum* and one strain each of *Treponema calligyrum*, *Treponema microdentium*, *Treponema mucosum*, and *Spirochæta refringens*. The results may be summarized as follows:

1. In the media containing glycogen and glucose, *Treponema microdentium* did not grow as vigorously as in other sugar media, and an earlier degeneration set in. One strain of the *pallidum* and the *calligyrum* and *mucosum* showed a poor growth in the glycogen medium. Similarly, there was little growth in the second transfer of these spirochetes in the glucose medium. The growth of the spirochetes in the media containing carbohydrates other than those just mentioned was generally good, and no difference could be distinguished between these and the control cultures without any carbohydrate. The only phenomenon which might be interpreted as indicating

a favorable influence of these media upon growth was the abundant growth of the *mucosum*, which showed uniform length, regular curves, and active motility somewhat better than in the sugar-free medium.

2. The height of acidity was found in the cultures containing glycogen and glucose in the *microdentium*, amounting to 0.1 N 4.8 cc. for 10 cc. of the fluid culture. In the other sugar media the acidity varied between 0.1 N 2 cc. and 0.1 N 3.2 cc. for the same amount. In the control cultures, the acidity fluctuated from 0.1 N 0.8 cc. to 0.1 N 2 cc. There was no visible alteration in the appearance of the media after the spirochetes had grown for 3 or 4 weeks. In the case of *Treponema microdentium*, a slight opalescence developed in the glycogen and glucose media after several weeks' standing, but there was no precipitation or coagulation of the proteins of the culture media.

3. There was no unusual morphological change in the spirochetes grown in the media containing any of the carbohydrates employed. The only phenomena which should be mentioned are (*a*) the frequent presence of the terminal appendages (or projections) in the *refringens* and in most of the *pallidum* strains, and (*b*) the appearance of minute, refractile spherical bodies along the side of the spirochetes in the *microdentium* cultivated in the glucose or glycogen media. Judging from the earlier degeneration of the species in the above mentioned media, these peculiar bodies may be interpreted as indicating a phase of plasmoptysis associated with the unfavorable surroundings prior to degeneration. Experimental evidence was not found for considering these spherules as a resistant or spore form of the spirochete.

In conclusion I wish to express my indebtedness to Dr. Hideyo Noguchi for his assistance and advice.

THE ABSORPTION OF FAT IN PARTIALLY, AND IN COMPLETELY DEPANCREATIZED DOGS

By CHARLES W. McCLURE, M.D., BETH VINCENT, M.D., AND JOSEPH H. PRATT, M.D.

(*From the Laboratory of Medicine, Harvard Medical School, Boston.*)

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For more than 50 years physiologists have studied the relation of the pancreas to the absorption of fat from the intestine, but the problem still remains unsolved. Many studies have been made on animals to determine whether the pancreatic juice is essential to the absorption of fat, or whether fat absorption is regulated by an internal secretion of the pancreas.

In 1856 Claude Bernard (1) presented detailed evidence for his claim that the pancreatic juice was of great importance in the process of digestion and in the absorption of fat by the intestines. In two dogs out of a series of ten, he found, after injecting melted suet or other substances into the main pancreatic duct, that the stools became very fatty. From the 11th to the 15th day one of these dogs passed large amounts of clear fat. In the 3rd week the feces contained less fat, and when the animals were autopsied it was found that the pancreatic juice was again discharging into the intestine.

The importance of the experiments of Claude Bernard was not appreciated for many years. The physiologists of his time, unable to confirm his observations, opposed the view that the pancreatic secretion was essential to the absorption of fat. Bernard replied to his opponents and showed that their experiments were without value, as they failed to exclude all the pancreatic secretion from the intestine. They remained unconvinced, and their conclusion, as expressed by Colin (2), that the pancreatic juice is not necessary for the digestion and absorption of the normal amount of fat, is the one held today by most investigators.

In 1890 Abelmann (3), working under Minkowski, studied fat absorption in dogs after partial and complete depancreatization. All communication between the pancreatic remnant and intestines was destroyed in the partially depancreatized dogs. These animals absorbed from 25 to 65.8 per cent of fats derived from olive oil, horse flesh, or butter. After the complete removal of the pancreas, the dogs were unable to absorb any of these fats, unless raw pancreas was fed, but they did absorb 28 to 90 per cent of the fat of milk.

The unabsorbed fats, however, were found to be split in the intestines. 35 to 85 per cent of the fat in fresh stools consisted of fatty acids and soaps. The fat in the small intestines contained 30 per cent of fatty acids and that in the colon 76 per cent, 12 hours after feeding neutral fat to a completely depancreatized dog.

The observations that fats were split but not absorbed, unless raw pancreas was added to the food, led Abelmann to conclude that: All fats, with the exception of milk fat, unquestionably require pancreatic juice to be absorbed. He explained fat absorption in the partially depancreatized dog by assuming that the agent effecting absorption is carried to the lumen of the intestine in some way as yet unknown.

Sandmeyer (4) reported finding in the stools of a partially depancreatized dog a greater amount of fat than had been fed. The pancreatic remnant in this dog consisted of a portion of the processus lienalis 3 cm. in length. A persistent glycosuria developed 1 month after the operation. Consecutive metabolism experiments were made throughout a period of 5 months. The percentages of fat lost in the dog's stools varied from 9.1 to 102 per cent in twenty-six experiments; from 104.6 to 111.2 per cent in eight; and from 122.08 to 163.5 per cent in four.

Rosenberg (5) separated the pancreas from the duodenum in a dog with the object of shutting out all the pancreatic secretion from the intestine. For a time the absorption of fat remained normal, but within 2 months a slight reduction occurred. In a series of fifteen experiments the absorption of fat averaged about 85 per cent and only once fell as low as 64 per cent.

In 1906 Lombroso (6), working with dogs, found that a large percentage of fat was absorbed, after all direct communication between the pancreas and duodenum was supposed to have been removed. This was also true, if the pancreas was extirpated except for a small portion which discharged its juice through a permanent fistula in the abdominal wall. When complete depancreatization had been effected, there was either slight or no absorption of fat. He concluded from his findings that the absorption of fat from the intestine is governed by an internal secretion of the pancreas.

Niemann (7) observed good absorption after tying the ducts. In five experiments on one dog 91.2 to 98.7 per cent of the fat in the food was absorbed; in three experiments on a second dog, 85.6 to 98.5 per cent.

Burkhardt (8) concluded from experiments on one dog that fat absorption was not regulated by an internal secretion of the pancreas. He extirpated all of the pancreas except the processus uncinatus. This portion was transplanted under the skin and a cutaneous pancreatic fistula was produced.

In the first metabolism experiment the animal was allowed to lick the fistula and in this way obtained pancreatic secretion. 80 per cent, or 47 gm., of the fat ingested was absorbed. In a second experiment in which licking the fistula was prevented, only 13 per cent, or 8 gm., of the fat was taken up by the intestine.

The findings in this pair of experiments were not verified in two succeeding

pairs of experiments. In these, no appreciable difference occurred in the fat absorption whether the dog did or did not lick the fistula.

Fleckseder (9) made a similar study. The splenic portion and the upper part of the body of the pancreas were extirpated. The remainder of the gland was separated from the duodenum and the main duct and surrounding pancreatic tissue transplanted into the abdominal wall, so that the secretion discharged externally. In only one experiment was there poor absorption (20 per cent) of fat, and later the same animal absorbed a much larger percentage (65 per cent), although it was prevented from obtaining any pancreatic juice. In one metabolism experiment a dog was allowed to lick the fistula, and less fat was absorbed than in the experiments when all pancreatic secretion was withheld. He concluded that the power of absorption of fat by the intestinal mucous membrane is chiefly dependent on an internal secretion of the pancreas.

Lombroso (10) in his later investigation repeated Burkhardt's work and obtained contradictory results. Sometimes more fat was absorbed when the dog was free to lick the pancreatic secretion from the permanent fistula and sometimes less. The absorption of fat in the animals with a subcutaneous graft varied from 48.5 to 80.3 per cent.

Pratt, Lamson, and Marks (11) in experiments on a series of five dogs found that when the pancreas was entirely separated from the duodenum, serious disturbance of fat absorption took place. The percentage of fat taken up by the intestine ranged from 4.8 to 76.6.

Jansen (12) partially extirpated the pancreas of a dog and transplanted the remainder under the skin. In three experiments the animal absorbed 74.9 to 76.4 per cent of the fat ingested. After the removal of the pancreatic graft the fat lost in the feces ranged from 15 to 156 per cent.

Visentini (13) devised a method of determining at autopsy whether or not pancreatic juice had been secreted into the intestine. In five dogs in which he had succeeded in permanently excluding all pancreatic secretion after ligature and resection of the ducts, the fat absorbed ranged from 28.7 to 44.0 per cent. In four dogs in which the corpus pancreatis and processus uncinatus were extirpated, the smallest percentage of fat absorbed was 8.7, and the largest was 25.7.

Much of the experimental work summarized above is open to criticism. There are three chief sources of error.

1. Failure to exclude all the pancreatic juice from the intestine. The difficulty of excluding permanently the pancreatic juice was shown clearly by Claude Bernard. Hess (14) thought that this was chiefly due to the presence of accessory ducts which were left untied. He showed that dogs might have three or four pancreatic ducts. This anomaly is probably much less common than Hess believed. Visentini failed to find more than two in a series of twenty dogs.

In 1909, Pratt, Lamson, and Marks showed that after the pancreatic ducts were tied and cut between double ligatures a sinus quickly formed between the main duct and the lumen of the intestine. Visentini in a careful investigation has demonstrated that after tying and resecting the two excretory ducts the flow of pancreatic juice may frequently be reestablished by the formation of a sinus.

2. Absorption experiments of too short duration. Abelmann's experiments lasted only 1 day and Burkhardt's 2 days. Some of Jansen's experiments were of only 1 and 2 days' duration. It is now generally recognized that trustworthy results cannot be obtained in such a short time.

3. No intervening period between experiments. Lombroso in his earlier studies often began a second experiment the same day that the preceding one was finished. We have found that dogs may pass stools containing the residue from a single meal for a period of 2 to 3 days. Therefore, in spite of the use of carmine to demarcate the stools, the possibility of the collection, during the second experiment, of feces which belonged to the first cannot be denied. In the final results this error would produce too high a figure for fat absorption in the first experiment, while the figure for fat absorption in the second experiment would be too low. This source of error can account for the results obtained by Burkhardt in his first two metabolism experiments, an explanation supported by the fact that in the two experiments the percentages of fat in the feces were nearly the same (23.8 and 26.3 per cent), but the amount of dried stool was nearly four times as great in the second as in the first.

In the animals studied by Pratt, Lamson, and Marks, rapid and progressive atrophy of the pancreas occurred. Pratt and Spooner (15) found that the power to assimilate glucose was rapidly reduced after tying the ducts, a fact which indicated damage to the internal function of the pancreas. Hence it might be asserted that the experiments of Pratt, Lamson, and Marks in which they found poor fat absorption after separating the pancreas from the duodenum did not disprove Lombroso's theory that the absorption of fat is largely due to some internal function of the pancreas. It should be pointed out, however, that Pratt and his coworkers found that the disturbance in fat absorption developed as soon as pancreatic secre-

tion was absent from the intestine while it was several weeks before there was a marked drop in the power to assimilate glucose.

Lombroso and Fleckseder published accounts of experiments which showed that the fat was fairly well absorbed when a subcutaneous pancreatic graft was made which was allowed to secrete externally, although the remainder of the gland had been extirpated. If the ducts secrete freely, then the degeneration of the gland tissue from back pressure does not occur. According to the view held by Pratt, Lamson, and Marks, it would make no difference in the absorption of fat whether the gland underwent degeneration or remained intact, provided the pancreatic juice did not enter the intestine.

It is a difficult procedure to remove all the pancreatic tissue from the wall of the duodenum, but to explain Lombroso's and Fleckseder's results by ascribing them to technical errors on their part would be unfair until their experiments had been repeated and refuted.

After complete extirpation of the pancreas it is held that little, if any fat is absorbed, except milk fat. This conclusion is based on the experiments of Abelmann and Lombroso. If true, it furnishes strong support for the theory that the presence of pancreatic tissue in the body, even when not connected with the intestine, aids in some way the absorption of fat. In all the experiments of Pratt, Lamson, and Marks, in which all pancreatic juice was excluded from the intestine, some absorption of fat took place. In only one experiment was as little as 4.8 per cent absorbed. If no absorption of fat occurs when the pancreas is totally removed, then the experiments published from this laboratory would indicate either that an internal pancreatic function regulated fat absorption, or that the presence of pancreatic tissue in the body in some other way affected the power of the intestinal wall to take up the fat. Abelmann, Rosenberg, Pflüger (16), and Burkhardt held that the pancreatic juice, after it is shut out from the duodenum, continues to form, but is taken up by the blood and carried to the other digestive glands and by them secreted into the intestines. Experiments by Lombroso overthrew this theory.

The first part of the present study deals with the absorption of fat in dogs with subcutaneous transplants of the pancreas which discharge their secretion externally. In the second part the results of experiments on completely depancreatized dogs are presented.

Methods.

Partial Pancreatectomy and the Formation of a Cutaneous Pancreatic Fistula.—Ether anesthesia was used in all the operations. Before etherization a subcutaneous injection of morphine was given. The processus lienalis and corpus pancreatis were completely extirpated, and great care was taken to remove from the duodenal wall every bit of adherent pancreatic tissue. The processus uncinatus of the pancreas was then freed from all its attachments except where the blood vessels entered at the lower end. Leaving these vessels intact, the major portion of the processus uncinatus was transplanted under the skin of the abdominal wall. This was accomplished by first incising the parietal peritoneum about 3 cm. to the right of the median abdominal incision. Then by blunt dissection the muscles were penetrated and a space for the pancreatic remnant was made in the subcutaneous tissue. After incising the skin a hemostat was passed through this space into the abdomen. By means of the hemostat the pancreatic remnant was drawn into the subcutaneous space. The distal end of the remnant was drawn through the incision to a little beyond the level of the skin. It was now fastened by a few fine stitches to the edge of the peritoneum and the skin.

This method was modified in Dog 4. The subcutaneous transplant consisted of the processus uncinatus and that portion of the body of the pancreas containing the main duct. The duct was preserved intact by excising that portion of the duodenal wall which contained the opening of the duct. The blood vessels entering this portion of the intestine were preserved. The portion of the pancreatic remnant containing the duct was made the distal end of the subcutaneous transplant.

Extirpation of the Subcutaneous Transplant.—In a second stage of the experiments the pancreatic remnant was completely extirpated. The abdominal portion of the remnant was first removed. This was done through an opening in the abdomen just to the right of the original median incision. Care was taken to close the opening made in the peritoneum. The remainder of the pancreatic transplant was removed through a skin incision.

Food.—The food consisted of a mixture of six parts of finely chopped,

boiled horse flesh, three parts of cracker dust, two parts of butter, and four parts of water. The butter was melted in boiling water and then intimately mixed with the solid ingredients. Fresh food was prepared for each feeding.

Metabolism Experiments on Dogs with a Subcutaneous Pancreatic Graft.—A period of from 5 to 7 days elapsed after operation before the dogs would take food readily. The experiments were then begun. A meal of milk was given 24 hours previous to the beginning of the experiment. This produced a thin, light colored stool quite different in appearance from the more formed deep yellow or gray stools resulting from the solid food of the metabolism experiment. 20 to 30 gm. of powdered charcoal were intimately mixed with the food of the first meal of each experiment. The resulting stools were very dark gray or black and easily separated from feces derived from other food. Feedings were made once daily over a period of 4 or 5 days. No food was given during the 48 hours succeeding the last meal of an experiment. Then bread and milk mixed with 20 to 30 gm. of charcoal were given. This permitted the collection of all feces belonging to the metabolism experiment. Each animal was kept in the same metabolism cage throughout an experiment except at the time of feeding, when it was transferred to a clean cage.

Metabolism Experiments on Completely Depancreatized Dogs.—These dogs received no food for 24 hours prior to the operation for the removal of the subcutaneous pancreatic remnant. Experiments were begun the next day after this operation. With the exception of this preliminary period of starvation the technique of the experiments was the same as described for partially depancreatized dogs. After the completion of one metabolism experiment a period of not less than 3 days elapsed before beginning another experiment.

Preservation of Specimens for Analysis.—One-fifth of the amount of food mixture eaten at each meal was preserved for analysis. It was mixed with 95 per cent alcohol containing 1 to 2 per cent by volume of hydrochloric acid and dried to constant weight on a water bath at 50–55°C. After drying, the food was finely triturated in a mortar and preserved in sealed jars. The same procedure was carried out with the stools, except that the entire amount of feces was preserved.

Analytical Methods.—Nitrogen was determined by the Kjeldahl method. Specimens were analyzed in duplicate. 0.5 to 1 gm. was used in each determination.

Fat was determined by the Folin-Wentworth (17) method. Specimens were analyzed in duplicate. 2 to 3 gm. of sieved material were used for each analysis. Extraction was carried on for a period of 20 hours. This method was not devised for the determination of the fat content of foodstuffs. However, we found that the same results were obtained by drying the fat, extracted by this method from our food mixture and from milk at 95°C., as when dried to constant weight at 55°C.

Interpretation of Findings.—The factors to be considered in comparing the results of absorption experiments are the percentage and weight of the fat absorbed in relation to the amount ingested and the weight of the animal. In the past, workers in this field have drawn their deductions from the percentages of absorption of the fat ingested. This method of expressing results does not tell the whole story regarding the fat absorbed. A relatively high percentage of absorption may represent but a small amount of actual fat, while a lower percentage may represent a great deal more fat. Not only should the percentage of absorption and the actual amount in grams be considered, but also the quantity of fat ingested. Within certain limits the larger the amount fed to an animal, the greater will be the amount absorbed by the animal. Size is an important factor if the number of grams of fat absorbed is to be compared in different dogs. In animals appearing to be about equally nourished the weight offers a simple and fairly reliable means for comparison of the size.

The metabolism in a completely depancreatized dog with the resulting diabetes is greatly disturbed. Animals after total pancreatectomy usually remain in suitable condition for experimentation but a short time. Because of this, it was necessary to work rapidly and without regard for certain factors which interfered somewhat with the accuracy of the results. Experiments were begun the day following the operation without regard to any possible postoperative effect upon absorption. No attempt was made to make the amounts fed uniform. The appetites of the dogs are often capricious.

In order to make certain of as large a food intake as possible during an experiment, the animals were given at a feeding as much food as would be taken.

ILLUSTRATIVE PROTOCOLS.

Dog 1.—Young adult female.

Dec. 21, 1915. Weight 14 kilos.

Dec. 22. Operation. Partial pancreatectomy. Corpus and processus lienalis, 20 cm. in length and weighing 16.5 gm., extirpated. Processus uncinatus, 9.4 cm. long, transplanted under skin of abdomen and a fistula made.

Dec. 25. Graft is noticed for the first time to be discharging. Dog appears well. No sugar in urine. Fed milk and ground pancreas.

Dec. 29. Dog ate ravenously. Some reddening of skin near opening in graft.

Dec. 30. Fistula closed.

Dec. 31. Dog appears ill. Tissues about graft distended; on opening with knife about 15 cc. of thin sanguineous fluid obtained which digests casein.

Jan. 2, 1916. On incising swollen area about graft 60 cc. of thin sanguineous fluid escaped.

Jan. 3. Weight 12.3 kilos. Fistula discharging thin watery fluid. Urine does not reduce Fehling's solution.

Jan. 5. *Metabolism experiment begun.* 559.8 gm. of food eaten, containing 30 gm. of charcoal.

Jan. 6. Weight 11.9 kilos. 647.5 gm. of food eaten. Large fatty stool. A few grams do not contain charcoal, and these are rejected. Weight of stool admixed with charcoal, 529.5 gm. Stools contain numerous muscle fibers, of which about half retain their striations.

Jan. 7. Weight 11.9 kilos. Dog seems well. Ate 948.6 gm. of food. Stools weigh 436 gm.

Jan. 8. Ate 948 gm. of food.

Jan. 9. Weight 11.6 kilos. No food fed. Fistula was discharging throughout experiment. Stools weigh 676 gm.

Jan. 10. Stools weigh 31 gm. Fed milk, bread, and 60 gm. of charcoal.

Jan. 11. Stool black with charcoal.

Jan. 19. Operation. Subcutaneous transplant removed. It measured 6.6 by 3.1 by 1.9 cm. At the first operation the graft was 9 cm. long, but fully 2 cm. of this was left projecting beyond the skin incision and was lost by desiccation and necrosis. Aside from some increase in firmness the pancreatic tissue of the graft appears normal.

Jan. 20. Dog died of peritonitis.

Metabolism Experiment. Jan. 5 to 8 (4 Days).

Food:	Moist weight	3,104 gm.	Dry weight 1,336 gm.
	Nitrogen:	71.5 "	5.35 per cent.
	Fat:	431.1 "	32.3 " "
Feces:	Moist weight	2,052.5 "	Dry weight 699.5 gm.
	Nitrogen:	39.0 "	5.6 per cent.
	Fat:	313.1 "	44.8 " "
Nitrogen absorbed:		45.37 per cent;	lost 54.63 per cent.
Fat "		27.37 " "	" 72.63 " "

Dog 2.—Adult male. Weight 15.1 kilos.

Feb. 11, 1916. Operation. Pancreas extirpated except processus uncinatus. This measured 5.6 cm. in length and was planted into the anterior abdominal wall and a permanent fistula made. The pancreatic tissue removed weighed 22 gm.

Feb. 12. Dog lively. Graft healthy.

Feb. 13. Fed 300 cc. of milk.

Feb. 14. Weight 13.85 kilos.

Feb. 15. Dog appears sick. Fistula not discharging.

Feb. 16. Dog active. Drank 500 cc. of milk. Fistula secretes a thin fluid admixed with blood. No sugar in urine.

Feb. 17. Weight 13.9 kilos. *First metabolism experiment begun.*

Feb. 18. Graft secreting actively.

Feb. 19. Weight 13.7 kilos. Feces partly formed, partly semisolid. They contain flakes resembling butter in appearance and consistency. Microscopic examination reveals many well preserved muscle fibers and fat globules.

Feb. 20. Dog lively. Fistula secreting freely.

Feb. 21. Weight 13.2 kilos. Stools slate-gray, fatty.

Metabolism Experiment 1. Feb. 17 to 21 (5 Days). Subcutaneous Graft. Extirpation of Remainder of Pancreas.

Food:	Moist weight	1,765 gm.	Dry weight 610 gm.
	Nitrogen:	37.9 "	6.22 per cent.
	Fat:	175.7 "	28.8 " "
Feces:	Moist weight	521.5 "	Dry weight 196 gm.
	Nitrogen:	16.9 "	8.6 per cent.
	Fat:	43.6 "	22.2 " "
Nitrogen absorbed:		21.12 "	43.96 " "
Fat "		132.1 "	75.20 " "

Feb. 22. No food given.

Feb. 24. Weight 12.96 kilos. Operation for removal of subcutaneous graft, which was firmly adherent to the surrounding tissues. Considerable blood lost

in cutting the adhesions, showing a large new blood supply to the graft. The excised pancreatic tissue was of normal appearance; the subcutaneous part was of firmer consistency than the intra-abdominal portion.

Feb. 25. Weight 11.8 kilos. Wound clean. *Second metabolism experiment started.* Ate 438.5 gm.

Feb. 26. Weight 11.2 kilos. Stool yellow, semisolid, and fatty, with rancid odor. Ate 9 gm. of food.

Feb. 27. Weight 11.2 kilos. Ate 91 gm. of food. Stool on microscopic examination found to contain many muscle fibers and much fat in the form of globules.

Feb. 28. Dog found dead in cage.

Autopsy.—No cause for death found. No pancreatic remnants in the abdomen or at the site of the pancreatic transplant in the abdominal wall. Stomach and small intestine empty. 58.5 gm. of fecal material were present in the colon and were added to the stools for analysis.

Metabolism Experiment 2. Feb. 25 to 27 (3 Days).

Food: Moist weight	539 gm.	Dry weight	160.0 gm.
Nitrogen:	10.88 "	6.8 per cent.	
Fat:	43.60 "	27.3 " "	
Feces: Moist weight	232.5 "	Dry weight	96.5 gm.
Nitrogen:	7.68 "	7.9 per cent.	
Fat:	23.85 "	24.7 " "	
Nitrogen absorbed:	3.20 "	29.41 " "	
Fat "	19.75 "	45.3 " "	

Dog 3.—Mar. 9, 1916. Weight 16.5 kilos. Operation. Subcutaneous transplant of processus uncinatus which was 10 cm. long. Remainder of pancreas, weighing 27 gm. extirpated.

Mar. 13. Good recovery from operation. Graft began to secrete.

Mar. 14. Weight 15.2 kilos. Dog lively. Fistula discharges a thin colorless fluid. *First metabolism experiment begun.*

Mar. 15. Weight 14.7 kilos.

Mar. 16. Weight 14.9 kilos. Stool semisolid, fatty, and contains a great number of muscle fibers and fat drops. Weight of feces 356 gm.

Mar. 17. Weight 14.9 kilos. Dog lively. Stools light yellow and of rancid odor; weight 411 gm.

Mar. 18. Weight 15.0 kilos. Fistula secreting. No food today. Stools weigh 173 gm.

Mar. 19. Stools 10 gm. Fed milk, bread, and charcoal.

Mar. 20. Weight 14.6 kilos. Stools black with charcoal.

Metabolism Experiment 1. Mar. 14 to 17 (4 Days).

Food:	Moist weight	2,110.5	gm.	Dry weight 765.0 gm.
	Nitrogen:	45.08	"	5.9 per cent.
	Fat:	215.88	"	28.2 " "
Feces:	Moist weight	1,161.5	"	Dry weight 417.5 gm.
	Nitrogen:	28.81	"	6.9 per cent.
	Fat:	164.29	"	39.4 " "
Nitrogen absorbed:		16.27	"	36.1 " "
Fat	"	51.6	"	23.9 " "

Mar. 21. Weight 14 kilos. Operation for removal of subcutaneous graft. The pancreatic remnant appeared normal.

Mar. 22. Weight 13.5 kilos. *Second metabolism experiment begun.* Ate 807.5 gm. of food. Dog appears in good condition but lies in cage.

Mar. 23. Dog lies in cage. Ate 77 gm. of food. Urine (480 cc.) reduced Fehling's solution. Stool 245 gm., semisolid, dark with charcoal. 3 gm. of stool not containing charcoal discarded.

Mar. 24. Dog much more lively. Stools weigh 143 gm.; the greater part admixed with charcoal. They are semisolid and microscopically show large numbers of well preserved muscle fibers and fat droplets.

Mar. 25. Dog very active. Stools weigh 284 gm.; diarrheal, light yellow.

Mar. 26. No food today. Stools weigh 113 gm.

Mar. 27. Weight 13.5 kilos. Feces 10 gm. Milk, bread, and charcoal fed. Dog appears well.

Metabolism Experiment 2. Mar. 22 to 25 (4 Days).

Food:	Moist weight	1,786.0	gm.	Dry weight 575.0 gm.
	Nitrogen:	34.5	"	6.0 per cent.
	Fat:	176.18	"	30.6 " "
Feces:	Moist weight	1,172	"	Dry weight 397.5 gm.
	Nitrogen:	25.21	"	6.3 per cent.
	Fat:	145.19	"	36.5 " "
Nitrogen absorbed:		9.29	"	26.9 " "
Fat	"	30.99	"	17.6 " "

Mar. 28 to Apr. 8. The dog became progressively weaker and after Apr. 2 was unable to stand. A severe and persistent glycosuria developed within 24 hours after the removal of the pancreatic remnants. Apr. 8. Killed with chloroform. No pancreatic tissue found in the abdominal cavity.

Dog 4.—Adult male dog.

Apr. 13, 1916. Weight 14.4 kilos. Entire pancreas removed except the processus uncinatus and lower part of the corpus pancreatis, which were transplanted with the main duct into the subcutaneous pocket.

Apr. 16. Recovery uneventful. No sugar in urine. Fed milk.

Apr. 17. *First metabolism experiment begun.* Weight 13.2 kilos. Fistula

secretes a clear colorless fluid. Ate 819 gm. of food. It contained 35 gm. of charcoal. Dog lively.

Apr. 18. Dog vomited; experiment stopped.

Apr. 19. Fed milk. Dog appears well.

Apr. 20. *First metabolism experiment recommenced.* Weight 13.4 kilos. Fistula discharges copiously. Ate 745.5 gm. of food.

Apr. 21. Food 375 gm.

Apr. 22. Dog well. Food 748 gm. Stool 163 gm. The skin about the fistula is reddened and excoriated.

Apr. 23. Dog lively. Fistula secreting. Food 799 gm. Stool 202 gm.; light yellow, and semisolid; contains abundance of muscle fibers and fat droplets.

Apr. 24. Weight 13.35 kilos. No food today. Stool 155 gm.

Apr. 25. Stool 25 gm. Food: milk, bread, and charcoal.

Apr. 26. Part of stools contains no charcoal. This portion weighs 116 gm. and was saved for analysis.

Metabolism Experiment 1. Apr. 20 to 23 (4 Days).

Food:	Moist weight	2,668 gm.	Dry weight, 1,052.5 gm.	
	Nitrogen:	46.31 "	4.4 per cent.	
	Fat:	312.80 "	29.7 "	"
Feces:	Moist weight	781.0 "	Dry weight 260.0 gm.	
	Nitrogen:	16.12 "	6.2 per cent.	
	Fat:	80.42 "	30.9 "	"
Nitrogen absorbed:		30.19 "	65.2 "	"
Fat "		232.38 "	74.3 "	"

Apr. 27. Food: milk.

Apr. 28. Weight 12.3 kilos. *Second metabolism experiment started.* Fistula secreting actively. Throughout the experiment the dog wore a muzzle covered with tin in order to prevent licking of the fistula. The pancreatic transplant secreted freely.

Apr. 30. Stools are semisolid. Microscopically there are many well preserved muscle fibers and fat droplets.

May 2. Weight 12.9 kilos. No food.

May 3. Stool 197 gm. Dog well. Fed milk, bread, and charcoal.

Metabolism Experiment 2. Apr. 28 to May 1 (4 Days).

Food:	Moist weight	3,582 gm.	Dry weight 1,495 gm.	
	Nitrogen:	78.49 "	5.3 per cent.	
	Fat:	488.57 "	32.7 "	"
Feces:	Moist weight	2,018 "	Dry weight 713.5 gm.	
	Nitrogen:	43.74 "	6.1 per cent.	
	Fat:	257.65 "	36.1 "	"
Nitrogen absorbed:		34.75 "	44.3 "	"
Fat "		230.92 "	47.3 "	"

May 5. Weight 12.00 kilos.

May 7. Fistula secretes 0.5 cc. of fluid per minute. One drop of the fluid digests 5 cc. of 1 per cent casein in 4 hours.

May 8. Weight 11.35 kilos. Dog is growing weaker. He eats ravenously.

May 8 to 19. Dog remains well. He is fed raw pancreas of three pigs, with chopped horse flesh and 500 cc. of milk daily. A gain in weight from 11.35 to 12.5 kilos occurred. The secretion of the fistula has gradually diminished.

May 19. Weight 12.5 kilos. The dog is in good condition but looks thin. Removal of the subcutaneous graft.

May 20. Weight 11.6 kilos. Dog lively and appears well. Urine, 370 cc., contains 18.5 gm. of glucose. *Third metabolism experiment started.*

May 21. Weight 11.15 kilos. Dog apathetic, but does not appear sick. Ate 441 gm. of food. No stools.

May 22. Food 380 gm. Stools have rancid odor and all contain charcoal; weight 131 gm.

May 23. Weight 11.15 kilos. Dog lively. Food 285 gm. Feces weigh 222 gm., 110 gm. of which contain much charcoal. On microscopic examination found to contain many muscle fibers and fat droplets.

May 24. Food 673 gm. Feces 266 gm.; partly formed, partly semisolid.

May 25. Weight 10.65 kilos. No food allowed. Stools 358 gm.

May 26. Fed milk, bread, and 30 gm. of charcoal. Stools 94 gm.

Metabolism Experiment 3. May 20 to 24 (5 Days).

Food:	Moist weight	1,836 gm.	Dry weight 717.5 gm.	
	Nitrogen:	37.88 "	5.3 per cent.	
	Fat:	213.67 "	29.8 "	"
Feces:	Moist weight	1,071 "	Dry weight 413 gm.	
	Nitrogen:	28.13 "	6.8 per cent.	
	Fat:	155.45 "	37.6 "	"
Nitrogen absorbed:		9.76 "	25.8 "	"
Fat	"	58.22 "	27.2 "	"

The dog lost 600 gm. during this experiment. He emaciated rapidly and became progressively weaker.

May 27. Weight 10.6 kilos. All the stools contain much charcoal. Urine, 650 cc., contains 81.25 gm. of glucose. *Fourth metabolism experiment started.* Lard fed instead of butter. Food 1,099 gm.

May 28. Weight 10 kilos. Dog stands steadily and seems well. Food 926 gm. Feces 409 gm.

May 29. Weight 9.9 kilos. Dog appears well. Food 747 gm. Feces 227 gm.; light yellow, semisolid.

May 30. Weight 9.9 kilos. Food 677 gm. Feces 532 gm.

May 31. Weight 9.75 kilos. Dog found lying in cage, but arises when approached; unsteady on his feet. Feces 440 gm.

June 1. Weight 9.2 kilos. No food today. Dog very weak. Stool 70 gm.

Metabolism Experiment 4. May 27 to 31 (5 Days).

Food:	Moist weight	3,449 gm.	Dry weight	1,402.5 gm.
	Nitrogen:	73.35 "	5.2 per cent.	
	Fat:	483.72 "	34.5 " "	
Feces:	Moist weight	1,678 "	Dry weight	664 gm.
	Nitrogen:	37.18 "	5.6 per cent.	
	Fat:	304.31 "	45.8 " "	
Nitrogen absorbed:		36.17 "	49.3 " "	
Fat "		179.41 "	37.1 " "	

June 2. Weight 9.1 kilos. Stands very unsteadily. Lies in cage unless some one approaches him. Etherized. No pancreatic remains found in the abdomen.

Dog 5.—Female pup.

Mar. 16, 1916. Weight 4.8 kilos. Entire pancreas except processus uncinatus removed. The latter was left *in situ*. It measured 5 by 2.5 cm. The pancreatic tissue is separated from the duodenum by mesentery.

Mar. 17. Dog lively. Fehling's solution not reduced by urine.

Mar. 23. Dog drinks milk and eats meat; seems well.

Mar. 26. Weight 3.5 kilos.

Mar. 27 to Apr. 8. Fed raw pancreas ground up with cooked horse flesh and milk.

Apr. 9. Fed only milk.

Apr. 10. Weight 4.8 kilos. *First metabolism experiment begun.* Dog in good condition. Food eaten 209.5 gm., containing 20 gm. of charcoal.

Apr. 11. Food 558 gm. Large part of feces contains charcoal; weight 108 gm. Portion free from charcoal easily separated.

Apr. 12. Food 651.6 gm. Feces 122 gm.; dark gray, formed, and found to contain on microscopic examination large amounts of muscle fibers and fat droplets.

Apr. 13. Food 239 gm. Feces 158 gm.; semisolid, yellow, fatty.

Apr. 14. Food 260 gm. Feces 123 gm.

Apr. 15. Fed 300 cc. of milk with charcoal. Feces 57 gm. Dog lively.

Apr. 16. Feces, 22 gm., unmixed with charcoal.

Apr. 17. Weight 4 kilos. Fed raw pancreas with chopped cooked horse flesh and milk. All the feces contain charcoal.

Metabolism Experiment 1. Apr. 10 to 14 (5 Days).

Food:	Moist weight	1,709 gm.	Dry weight	550 gm.
	Nitrogen:	32.01 "	5.8 per cent.	
	Fat:	150.9 "	27.4 " "	
Feces:	Moist weight	592 "	Dry weight	220 gm.
	Nitrogen:	14.98 "	6.8 per cent.	
	Fat:	72.07 "	32.8 " "	
Nitrogen absorbed:		17.98 "	56.2 " "	
Fat "		77.8 "	51.6 " "	

June 1. Weight 4.8 kilos. Urine contains sugar for the first time. Since Apr. 15 the dog has been given raw pig's pancreas daily.

June 29. Weight 4.1 kilos. Urine reduces Fehling's solution.

July 26. Found dead this morning. Weight 2.9 kilos.

Autopsy.—Dog greatly emaciated. All internal organs seem free from disease. A piece of tissue resembling a lymph node lies near the lower end of the duodenum in the mesentery. It is about 1 cm. in diameter and the thicker portion is surrounded by a zone of thinner tissue making the whole mass measure 3 by 1 cm. On histological examination this was found to be the atrophied and sclerosed pancreatic remnant.

Fat Absorption in Partially Depancreatized Dogs.

Metabolism experiments were done on four partially depancreatized dogs with subcutaneous transplants, which discharged the pancreatic juice externally. The animals were free to lick their fistulas and thus ingest pancreatic juice. How much pancreatic secretion was obtained in this way could not be determined. The amounts and percentages of fat absorbed are given in Table I.

TABLE I.

Fat Absorption in Partially Depancreatized Dogs in Which All Pancreatic Juice Was Excluded from the Intestine.

Dog.	Weight.*	Fat ingested.		Fat absorbed.			Remarks.
		Total amount.	Amount per kilo.	Total amount.		Amount per kilo.	
	kg.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	
1	11.9	431.12	36.1	118.0	27.37	9.88	Subcutaneous transplant; remainder of pancreas extirpated.
2	13.5	175.68	13.0	132.1	75.2	9.78	
3	14.9	215.88	14.5	51.6	23.9	3.47	
4	13.4	312.8	23.38	232.38	74.3	17.4	
5	4.4	150.9	34.28	77.8	51.6	17.66	Processus uncinatus left *in situ;* remainder of pancreas extirpated.

*The weights given are the averages of the daily weights of each dog.

Dogs with functionating subcutaneous grafts with the external secretion discharging freely may absorb no more fat than a dog in which the ducts are occluded and the gland undergoing rapid and progressive atrophy. This is seen by comparing the results of the experiments on the first four dogs (Nos. 1, 2, 3, and 4) with those on Dog 5, presented in Table I. In Dog 5 the processus uncinatus

was left in the abdomen and the remainder of the pancreas removed. There was no communication between the pancreatic remnant and the lumen of the duodenum; the dog received neither pancreatic juice nor pancreas in its food, and yet this animal absorbed 51.6 per cent of the fat ingested. The mean average of the percentages absorbed by the other four animals is 50.19 per cent. Dog 5 absorbed 17.66 gm. of fat per kilo of body weight. 17.38 gm. was the maximum per kilo absorbed by any of the other animals, in the tabulated results.

The fact that the fat absorption in the dogs with atrophied pancreas, studied by Pratt, Lamson, and Marks, was less on the average than in our dogs with pancreatic transplants which were discharging pancreatic juice freely, would seem at first sight to support Lombroso's view that absorption varied with the condition of the pancreatic remnant. But in Dog 5 with atrophying pancreas, as we have shown, the absorption was equal to that in the dogs with subcutaneous transplants that were not undergoing rapid atrophy. The same food mixture was given to all the animals in the present investigation. In the earlier study of Pratt, Lamson, and Marks a different food mixture was fed and it may have been less easily absorbed. However, there is no doubt that different animals when deprived of their pancreatic juice vary greatly in their power to absorb fat, and furthermore, that there is a great variation in the same animal on the same diet at different times. This was shown in the experiments of Pratt, Lamson, and Marks.

In the present study Dogs 2 and 4 absorbed the relatively large percentages of 75.2 and 74.3 per cent of the fat ingested, while Dogs 1 and 3 absorbed but 27.37 and 23.9 per cent.

Much more secretion flowed from the cutaneous fistula in Dog 4 than from the fistulas of the other animals. A proteolytic ferment was demonstrated in the secretion. The dog was seen to lick the fistula frequently and it is probable that the animal ingested more secretion than did the other dogs. Nevertheless, the percentage of fat absorbed by Dog 4 was no greater than that absorbed by Dog 2.

The number of grams of fat absorbed per kilo of body weight varied in the different dogs. No relation existed between the number of grams of fat ingested and the amount absorbed.

Burkhardt, Fleckseder, and Lombroso carried out metabolism experiments on dogs with pancreatic cutaneous fistulas. Experiments were made during two periods, one in which the fistulas were licked and one in which the licking was prevented. Conflicting results were obtained.

Fleckseder and Lombroso concluded from their experiments that, since it made little difference in the absorption of fat whether or not the animal obtained the pancreatic secretion from the pancreatic transplant, the absorption must be governed by an internal function of the pancreas. We have repeated the experiments on one dog. The results are given in Table II.

TABLE II.

Fat Absorption in Dog 4 with and without Licking of Pancreatic Juice.

Experiment No.	Date.	Weight.*	Fat ingested.		Fat absorbed.			Remarks.
			Total amount.	Amount per kilo.	Total amount.		Amount per kilo.	
		kg.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	
1	Apr. 20 to 23	13.37	312.80	23.38	232.38	74.30	17.38	Fistula licked.
2	" 28 " May 1	12.60	488.57	38.77	230.92	47.3	18.32	Licking prevented; dog muzzled.

* The weights given are the averages of the daily weights during each experiment.

Dog 4 in the first metabolism experiment was permitted to lick the fistula and thus obtain the pancreatic juice. In the second experiment the dog was prevented from licking the fistula by means of a muzzle covered with tin. The fistula in this animal continuously secreted enough fluid to bathe the entire ventral parts of the abdomen and chest. The skin over these areas showed evidences of being digested. The secretion from the fistula contained trypsin.

In the first experiment 74.3 per cent of the ingested material was absorbed. After muzzling the dog the absorption dropped to 47.3 per cent. This increased loss in the feces of 27 per cent of the fat ingested suggests that the pancreatic secretion increased the amount of fat absorbed. This view is supported by the fact that the feces contained 5.2 per cent more fat. The total amount of fat absorbed, however, was nearly the same in the two experiments, and the amount of fat absorbed per kilo was actually greater when the dog was prevented from licking the fistula. As the dog was not muzzled

until the experiment was begun, it is possible that pancreatic secretion ingested previously influenced somewhat the absorption of fat.

It would not be surprising if the pancreatic secretion introduced *per os* had increased somewhat the absorption of fat, because Pratt, Lamson, and Marks observed a greater absorption after the feeding of pancreatic ferments to dogs in which the pancreas had been separated from the duodenum.

Lombroso believed that a definite relation existed between the state of preservation of the pancreatic remnant in his dogs and the amount of fat absorbed. This conclusion was based upon the finding of degenerated pancreatic remnants in dogs showing a poor fat absorption. But his own findings and those of other investigators do not support this view. In the first metabolism experiments following partial pancreatectomy, one of his dogs absorbed 46 per cent (48 gm.), and another dog absorbed none of the fat ingested. 2 weeks later the same animals absorbed 68 per cent (66 gm.) and 16 per cent (13 gm.). There is no reason to assume that the pancreatic remnant was in a better state of preservation 2 weeks after the first metabolism experiments.

Two dogs, reported by Rosenberg, absorbed from 49.06 to 84 per cent of the fat ingested, at a time when the pancreas had undergone almost complete fibroid degeneration. Sandmeyer studied the fat absorption in a partially depancreatized dog in which he believed no communication existed between the pancreas and duodenum. The percentages of fat lost in the stools of this dog varied from 9.1 to 163.5 per cent. The greatest fat losses were not confined to any one period, but occurred from time to time throughout the investigation. Hence, they could not be attributed to degeneration of the pancreatic remnant.

Dogs 1 and 3 absorbed but 27.37 and 23.9 per cent. These low percentages of fat absorption could not be ascribed to degenerative changes in the pancreatic remnants as maintained by Lombroso, because when these were removed at the second operation they were found to be well preserved.

Fat Absorption in Completely Depancreatized Dogs.

At a second operation the pancreatic remnant was removed from Dogs 2, 3, and 4. Fat absorption was then studied in these animals. The results are given in Table III.

TABLE III.

Fat Absorption in Completely Depancreatized Dogs.

Experiment No.	Dog.	Weight.*	Fat ingested.		Fat absorbed.		
			Total amount.	Amount per kilo.	Total amount.		Amount per kilo.
		kg.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*
2	2	11.4	43.6	3.82	19.75	45.3	1.73
2	3	13.4	176.18	13.11	30.99	17.59	3.05
3	4	11.2	213.67	19.00	58.22	27.24	5.17
4	4	10.1	483.72	48.22	179.41	37.1	17.88

* The weights given are the averages of the daily weights of each dog.

Dog 2 was in poor condition during the metabolism experiment and died before its completion. The animal ingested but 43.6 gm. of fat. Nevertheless, 45.3 per cent of this was absorbed.

Each dog absorbed a considerable amount of fat, although the percentages, the actual amount, and the number of grams per kilo of body weight varied much in the different animals and in the two experiments on the same animal. As the autopsies on these dogs showed that all pancreatic tissue had been extirpated, the experiments prove that fat may be absorbed from the intestine by depancreatized dogs in relatively large amounts, even when the fat fed is not in the form of an emulsion. Dog 4 absorbed 37 per cent of fat fed, chiefly in the form of lard, 9 to 12 days after the removal of all pancreatic tissue. In the other experiments butter was given. Since the publication of Abelmann's studies in 1890, it has been generally held that a depancreatized dog can absorb only the fat of milk.

A comparison of the fat absorbed during the stage of partial depancreatization and after complete pancreatectomy is given in Table IV.

After partial depancreatization Dog 2 absorbed 75.2 per cent of the fat ingested (Metabolism Experiment 1), while 45.3 per cent was absorbed after complete pancreatectomy. Results almost identical to these were obtained in the first and second metabolism experiments on Dog 4, although the transplant was still secreting in the second experiment. The only difference is that the dog obtained no secretion, as it was muzzled. It will be seen that Dog 2 absorbed a larger percentage of fat when completely depancreatized

TABLE IV.

Comparison of Fat Absorption before and after Complete Depancreatization.

Experiment No.	Dog.	Date.	Weight.*	Pancreatectomy.	Fat ingested.		Fat absorbed.			Remarks.
					Total amount.	Amount per kilo.	Total amount.		Amount per kilo.	
			kg.		*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	
1	1	Jan. 5 to 8	11.9	Partial.	431.12	36.10	118.0	27.4	9.88	Fistula licked.
1	2	Feb. 17 to 21	13.5	Partial.	175.68	13.0	132.1	75.2	9.78	Fistula licked.
2		" 25 " 27	11.4	Complete.	43.60	3.82	19.75	45.3	1.73	
1	3	Mar. 14 to 17	14.9	Partial.	215.88	14.5	51.6	23.9	3.47	Fistula licked.
2		" 22 " 25	13.4	Complete.	176.18	13.11	30.99	17.6	3.05	
1	4	Apr. 20 to 23	13.4	Partial.	312.8	23.38	232.38	74.3	17.4	Fistula licked.
2		" 28 " May 1	12.6	"	488.56	38.77	230.92	47.3	18.32	Muzzled.
3		May 20 " 24	11.2	Complete.	213.67	19.00	58.22	27.2	5.17	
4		" 27 " 31	10.0	"	483.72	48.22	179.41	37.1	17.88	

* The weights given are the averages of the daily weights throughout an experiment.

than did Dog 4 which possessed functionating pancreatic tissue. These findings are decidedly against Lombroso's theory that absorption is controlled by an internal secretion of the pancreas. Dogs 3 (Metabolism Experiments 1 and 2) and 4 (Metabolism Experiments 2 and 4) absorbed but 6.3 and 10.2 per cent less fat after complete depancreatization than after partial extirpation of the gland. Greater differences in the percentage absorption of fat in the same animal are reported by Lombroso, and Pratt, Lamson, and Marks, in their studies on partially depancreatized dogs.

The number of grams of fat absorbed per kilo of body weight varies in experiments on the same dog and on different dogs, as is shown in Table IV. Dog 2 absorbed much less fat after complete than after partial depancreatization. But it will be noted that much less fat was ingested. A similar result occurred in the first metabolism experiment following complete depancreatization in Dog 4.

The poorer absorption in these dogs may have been a result of the operation. The experiments were begun the day following. However, under the same experimental conditions Dog 3 did not show an appreciable increase in the fat lost in the feces. In the first experiment Dog 3 absorbed 3.47 gm. of fat per kilo of body weight. At this time the dog was partially depancreatized and was allowed to lick the secretion from the fistula. After complete depancreatization 3.05 gm. of fat per kilo were absorbed. After complete extirpation of the gland, Dog 4 (Metabolism Experiment 4) absorbed but 0.44 gm. less fat per kilo than after partial depancreatization (Metabolism Experiment 2). In the latter experiment the dog possessed a cutaneous pancreatic fistula, but was muzzled so that no pancreatic secretion could be ingested. In Metabolism Experiment 4, 0.5 gm. more fat was absorbed per kilo than in the first metabolism experiment, in which the dog licked the pancreatic fistula. These findings show that it is possible for a completely depancreatized dog to absorb as much fat per kilo of body weight as when the same dog possesses functionating pancreatic tissue which does not secrete into the intestines.

CONCLUSIONS.

It was found that dogs with a subcutaneous transplant secreting and discharging pancreatic juice externally absorbed no more fat than dogs in which the pancreatic remnant was undergoing rapid atrophy and sclerosis. Hence, the condition of the pancreatic tissue remaining in the body does not influence the amount of fat absorbed by the intestine.

The absorption of fat by the intestinal mucous membrane is always markedly disturbed when the pancreatic secretion is excluded from the intestine.

After the complete removal of all pancreatic tissue from an animal, the absorption of considerable amounts of fat can still take place.

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SOME FIELD EXPERIMENTS BEARING ON THE TRANSMISSION OF BLACKHEAD IN TURKEYS.

By THEOBALD SMITH, M.D.

(From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

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During the spring and summer of 1916 when the Department buildings and grounds were not yet available, a small piece of land on the campus of Princeton University was put at our disposal by the University authorities. On this land the experiments to be described were carried out. Although they are incomplete on account of restricted facilities, it seems desirable to publish them now, rather than wait another year, in order to stimulate others to utilize the more positive features of the work and thereby save a year in testing the final practicability of the suggestions made.

To obtain animals free from infection, for experimental purposes, it was necessary to rear them, as was done in 1913 and 1914, in the incubator and brooder.[1] To test again at the same time the presence or absence of the parasite of blackhead in eggs, these were obtained from three different farms on all of which the disease had prevailed during the preceding season, if we are to accept mortality as an indicator. The positive demonstration of blackhead on two of the farms was made by autopsies in the course of the season.

Since the artificial incubation and brooding of turkeys is not practised at the present time, a few details of the procedure as actually followed may not be amiss, although some of these details have been referred to in an earlier paper.[1] Just how far the precautions exercised will be necessary in practice must be worked out later. In the work before us, the main task was to obtain a lot of healthy animals. Any failure meant the postponement of further work for a whole year.

[1] Smith, T., *J. Med. Research*, 1915, xxxiii, 243.

After collection, the eggs were kept in a cool place and turned once a day. For transportation to Princeton, they were wrapped in cotton-wool, boxed, and carried in the hand. No time was lost in starting incubation. As a preliminary precaution, they were washed and gently scrubbed with warm water, dipped in warm 0.5 per cent mercuric chloride for 30 seconds, then washed in warm boiled water, and dried with sterilized towels. When placed in the incubator, April 21, 1 egg was 11 days old, 10 were 6 days old, 11 were 5 days old, 7 were 4 days old, 9 were 3 days old, 3 were 2 days old, and 1 was 1 day old. 1 was found cracked later on and rejected, leaving 41 eggs in all.

The incubator used was an ordinary one with a capacity of 144 eggs. The kerosene lamp was replaced by a Bunsen burner. Three shallow pans were supported on rods in the bottom of the egg chamber and in these water was kept throughout the period of incubation. Moisture was not supplied in any other way. The eggs were turned twice a day but were not otherwise handled. All the turkeys hatched on May 18 in the course of 24 hours.

From Source A, 24 eggs were incubated, 22 hatched. The remaining 2 eggs contained full grown embryos.

From Source B, 5 eggs were incubated, 2 hatched, 1 failed, and 2 were sterile.

From Source C, 12 eggs were incubated, 6 were sterile, 3 hatched. 2 had full grown embryos and 1 a partly macerated small embryo.

The results would perhaps have been better under other than improvised surroundings. The incubator temperature rose to 107° F. during the night of the 1st and 2nd day, and it was feared that the embryos had been destroyed. On the whole, the output was as large as could be handled under the circumstances.

During the 1st week four poults with weak legs were killed because they were unable to feed themselves properly. There were thus left twenty-three vigorous poults from forty-one eggs. On May 19, they were moved about a mile and placed in a brooder on a house porch, protected against rain, where they could be closely watched and properly fed. On June 1, brood and brooder were again carried some distance to an open, wire-screened shelter about 9 feet by 12 feet with a sloping canvas top to keep the rain off. This shelter

was placed on a lawn directly behind the laboratory building (Guyot Hall of Princeton University) where the Department was housed during the year. The southern exposure and proximity to the buildings made the shelter at times extremely hot during the summer. Rain was not entirely excluded since the screened sides were not protected. The position of the shelter was, however, such that it was protected from driving rains. On June 17, 30 days after the hatching, the brooder was removed from the shelter and from that time on the turkeys spent the day in a yard staked out with poultry wire which was occasionally shifted. The yard covered, roughly speaking, about one-eighth of an acre. Sparrows and song birds visited the grounds daily. During the night and on rainy days the turkeys were locked in the shelter.

Up to July 3, 46 days after hatching, the twenty-three turkeys had not had any illness. They were all vigorous and without visible deformities.

So far, the result of the work was clear. Twenty-three turkeys were carried along for more than 6 weeks without death or disease. This confirms on a larger scale the work done previously. It shows that healthy turkeys may be raised from the eggs of infected flocks when all older turkeys and poultry are kept away and the ground has not been used before. There is thus no evidence favoring the theory that the protozoan parasite can be transmitted in the egg. The experiment also shows that young turkeys are more resistant than is usually assumed. This flock was handled and moved about considerably and was not kept in a closed shelter at night after the 1st month. The want of resistance exhibited by young turkeys must, at least in part, be ascribed to latent infection waiting on some depression of vitality for multiplication in the tissues. In this resistance the food probably plays a considerable but as yet unmeasured part. As there was no latent infection or an infected environment to be dealt with, the food actually given and to be described may not be satisfactory in the presence of infection. It should, therefore, be understood that no claim is made for the food or treatment in warding off disease, as there was none to guard against. It was, however, necessary to provide the best food available to counteract the influences of confinement.

The poults were fed about four times a day during the 1st month. During the first 3 days in the brooder, they received bread, scalded milk, boiled egg, and some lettuce. Thereafter they were fed with a mixture prepared and sterilized in glass jars and made up as follows: 500 gm. of chick feed and 1,000 cc. of milk plus 5 eggs, mixed thoroughly after heating in a double boiler, and then autoclaved. In addition they received sour milk and lettuce. On warm days they were allowed to move about on the lawn and obtain some grass. The food mixture was changed from time to time both in amount and composition, by replacing an increasing amount of cooked food with raw chick feed, until a relatively small per cent of the food was cooked. Later, coarser grain was substituted. The sour milk was continued during the summer and fall.

Exposure to Domestic Fowls.

The statement has been made from time to time that the parasite of this disease may be carried and disseminated by poultry. No conclusive experiments for or against this supposition have been made. Nor is it likely that any one, or even several tests can decide the question. It is conceivable that fowls in one geographical section or fowls of a given breed may act as agents. The writer's experience in 1910,[1] when incubator turkeys kept near a poultry yard died of blackhead, made a test of this possible source of infection seem most urgent.

On July 3, four incubator turkeys were segregated in a second enclosure for an experiment to be described later. On July 14, when the remaining nineteen turkeys were 57 days old, they were divided into two lots of nine and ten respectively. The screened shelter was divided into two sections by a partition. Two doors led to two different yards, separated by a lane at least 5 feet wide.

Two yearling hens (White Leghorn), from a farm having no turkeys, were penned with one lot of nine turkeys from July 14 until August 14—a period of 31 days—when the experiment was closed. None of the turkeys showed any indications of disease during this period or at the end of the experiment. It is probable that certain nematodes (*Heterakis*) found in the ceca of several turkeys later on came from the poultry.

Two yearling hens were penned with the second group of ten turkeys from July 17 to August 14, a period of 28 days. No disease or minor ailment appeared among the turkeys during this time. The hens used came from a farm on which

only 8 turkeys survived out of a lot of 57. The hens, of which there were about a hundred, had been with the turkeys until 2 weeks before the test.

The negative outcome of these experiments does not, of course, prove that poultry does not transmit the protozoa of blackhead. It is, however, distinctly encouraging from the practical standpoint. Experiments similar to this should be made in different sections and with different breeds of fowls upon incubator turkeys.

Exposure to an Infected Adult Turkey on Non-Infected Ground.

On August 14, the nineteen turkeys which had passed through the exposure to poultry and remained well were divided into three lots. Two of these were sent to Farms A and C on which blackhead had prevailed during the season, in order to test their susceptibility at this time. A third lot of nine was kept in the original enclosure and an infected turkey-hen (No. 120) penned with them. This hen had been raised on Farm B in 1915. In 1916, she hatched and brooded nine poults among which there were deaths from blackhead when the poults were 19, 33, 34, 35, and 37 days old, respectively. She had not been in contact with young turkeys since July 3. Before placing her with the incubator turkeys, the feet were thoroughly scrubbed to remove all attached soil.

Within 17 days there were signs of disease in the enclosure. One (No. 137) died of blackhead on September 9, 26 days after beginning of exposure. No. 139 was very ill on September 12 and chloroformed. Pronounced lesions of the liver and ceca were found. On September 15, No. 132 was very ill and chloroformed. Nos. 140 and 141 which were ill for a week, beginning August 31, recovered and are still alive and of normal size.

Exposure to an adult turkey-hen thus led to disease within 17 days. Three died, two recovered, and four did not show any disturbance of health.

Exposure to Infected Adults on Infected Ground.

Six incubator turkeys were sent to Farm C, on August 14, to be placed on infected ground and come in contact with adult turkeys. Of these, one died on August 29, one on August 31, and one on September 1. Three survived but no information is at hand whether they were manifestly ill at any time.

Four incubator turkeys were exposed to a similar environment on Farm A, beginning August 14. One died on August 28, one on August 30, and one on September 3. One survived.

In all cases the dead birds were sent to the laboratory and the diagnosis of blackhead was confirmed by autopsy and microscopic examination of fixed and hardened tissue.

Exposure to Infected Young Turkeys.

On July 3, when the incubator turkeys were 46 days old, four were taken from the main flock to be penned with young infected birds.

In order to do this it became necessary to stake out a second enclosure or yard. It was separated from the first enclosure by a lane about 15 feet wide. A brooder was placed in it to afford shelter at night and during storms. The openings were enlarged and covered with wire netting to keep rats and other vermin out.

From a small brood on Farm B in which blackhead had appeared, two young turkeys (Nos. 154 and 155) were placed with the four incubator turkeys into the enclosure on virgin soil. On July 7, three young turkeys (Nos. 167, 168, and 169) from another brood on Farm B, in which blackhead prevailed, were placed into the same enclosure. On July 11, No. 168, being very feeble, was killed. The wall of one cecum was locally thickened but there was no necrosis or exudation into the lumen. Sections showed subsequently that the thickening was due to the parasite of blackhead. On August 14, 38 days after the poults had been penned together, No. 169 appeared droopy, and was chloroformed. The walls of one cecum over the distal half were greatly thickened but there was no exudate. The liver was permeated with yellowish foci from 2 to 6 mm. in diameter. Sections of the liver and ceca showed the usual picture of this disease.

On this day (August 14) the experiment was closed because the shelter was becoming too small and conditions were unsatisfactory. There were remaining at this time the three originally exposed birds, Nos. 154, 155, and 167, all apparently well, and four incubator turkeys, all of which had remained well to date. Of this latter group one was killed the following day. No traces of disease could be found.

This enclosure had thus harbored two diseased turkeys, one for 4 (No. 168), the other for 38 days (No. 169). It is impossible to state when the latter became actively diseased.

Unless our turkeys were naturally immune—an assumption disproved by experiments detailed above—the experiment shows that a prompt transfer of infection from the diseased young stock did not occur, in spite of the fact that these birds were housed in the confined space of a small brooder every night and associated in the enclosure during the day. This result is in harmony with the experiments of 1914.[1] Eleven incubator turkeys were at that time kept with eight acutely diseased turkeys in a small shelter at night and in the same open enclosure by day. Only one became acutely diseased and died.

The ultimate fate of this lot of turkeys is of no significance. Owing to the necessity for closing this experiment on account of inadequate shelter and grounds, the surviving turkeys were moved to grounds on Farm B which had been cleared of turkeys early in July. Several

died of blackhead here. It again became necessary to move the remainder and they were placed with those exposed to the adult hen already referred to. Only one survived and is now in fine condition.

DISCUSSION.

Before drawing any conclusions based on this season's field experiments, it will be necessary to point out several weak points. To make the exposure tests more conclusive, a certain number of incubator turkeys should have been kept as controls throughout the summer. The turkeys exposed to the hens should have been kept the rest of the season and not used in any subsequent tests. It was, however, impossible to provide the necessary shelters and runs.

To meet the first criticism on the absence of controls, it may be stated that this disease does not have a long period of incubation. A large percentage of poults die between the end of the 2nd and 3rd weeks of life. Thus during the present season, the first death among the brood of one hen on Farm B occurred in 18 days, among the brood of the second hen in 23 days.

The fact that all the incubator turkeys remained well throughout the time of association with hens and became diseased as late as 2 weeks after the exposure to actual infection had begun does not make the assumption that they had acquired disease from the hens probable. If they did contract the disease from them it required over 6 weeks from the beginning of the exposure to bring it out.

Table I gives the details of the two experiments.

More of the turkeys that had been exposed to hens not in previous contact with diseased turkeys died than of the other lot. A superficial study might give the impression that the second lot had obtained an immunizing dose from the hens previously exposed to blackhead, for six out of nine of the first lot and but three out of ten of the second lot became openly diseased. The difference is, however, due to the fact that the mortality of the turkeys penned with Turkey-hen 120 was only one-third. This low percentage may be ascribed to the relatively diluted infection due to one bird on virgin soil as compared with that existing on farms where the soil may be infected and where there are several adults.

TABLE I.

Turkey No.	Exposure to hens from July 14 and 17 to Aug. 14.	Exposure to blackhead infection on Aug. 14.	Result.
126		Farm A.	Died on Aug. 28. Blackhead.
129		" "	Lived.
130		" C.	Died on Aug. 29. Blackhead.
131		" "	" " " 31. "
132	Two hens from turkey-free farm.	Turkey-hen 120.	Chloroformed on Sept. 15. Blackhead.
133		Farm A.	Died on Sept. 3. Blackhead.
134		" C.	Lived.
135		" "	Died on Sept. 1. Blackhead.
136		Turkey-hen 120.	Lived.
124		" 120.	"
125		" 120.	"
127		Farm C.	"
128		" A.	Died on Aug. 30. Blackhead.
137	Two hens from farm infected with blackhead.	Turkey-hen 120.	" " Sept. 9. "
138		" 120.	Lived.
139		" 120.	Chloroformed on Sept. 12. Blackhead.
140		" 120.	Lived.
141		" 120.	"
142		Farm C.	"

SUMMARY.

It seems, therefore, pending further field tests on a larger territory, that the following facts are fairly well established by the above experiments.

1. Healthy turkeys may be raised in an incubator from eggs of infected birds. In the above experiments all remained well to August 14, the end of the hen exposure test, when they were 12 weeks and 4 days old. The first death occurred 2 weeks after the beginning of exposure to actual disease, when they were more than 14 weeks old.

2. Hens from a blackhead farm and from a farm free from turkeys did not convey the disease to the incubator turkeys on uninfected land.

3. The infection is either not transmitted at all or only under exceptional conditions by turkeys in the early acute stage. It is probably carried and shed by those birds which have successfully passed through an attack.

Any definite statement concerning the mode of transmission of the infection cannot be made. The vehicle is unknown. The nature of the disease makes it probable, however, that it is introduced with the food, that it lodges first in one or both ceca, and that fecal matter is the vehicle.

During the entire season, portions of the small intestines of all the turkeys that died or were killed were sectioned and examined both with reference to the possible presence of coccidia and of any preliminary stage of *Amœba meleagridis*. Sections were studied from the upper (duodenum), middle, and lower portions. A few coccidia cysts were found in two turkeys and are referred to more in detail elsewhere.[2] In a third turkey an intracellular parasite was seen which is very minute and which differs from those usually met with in birds. It is tentatively placed with the coccidia. It was not seen in any other case although searched for to obtain more material for study. It may be that we have an aberrant parasite to deal with which comes from the insects eaten and obtains lodgment in rare cases only. The existence of any earlier stages of the blackhead parasites in the small intestine whence they move down into the ceca is contradicted by the focal lesions found in the ceca and by the fact that in many cases only one cecum is attacked and this only in a single, restricted area. If the parasites multiply higher up we should expect both ceca to become infected.

There is some evidence pointing to a greater resistance of older birds than is usually presented by young turkeys. Thus, the total mortality (including those chloroformed while sick) following exposure on the infected farms and to the yearling turkey-hens was nine out of nineteen exposed, or a trifle less than 50 per cent. This figure is usually exceeded among those exposed immediately after hatching. The surviving turkeys are still well at the present time (January 1917).

The other kind of evidence is derived from the histological examination of the lesions in the liver and ceca. In these organs the process was rather early associated with extensive infiltration of roundish cells, while the tendency to necrosis was relatively slight. The lesions

[2] Smith, T., and Smillie, E. W., *J. Exp. Med.*, 1917, xxv, 415.

of the walls of the ceca were characterized by a marked thickening of the wall with little or no necrosis and exudation of fibrin.

In repeating tests of this kind it will be well for the experimenter to bear in mind that they are no longer laboratory experiments but conform to natural occurrences. The infection is not isolated in pure culture but is associated in the body of the carrier with unknown factors, both normal and pathological. The virus, if such it may be called, may be mixed with different injurious agents in different localities. It may be favored by various protozoan or higher parasites accidentally present, and by digestive disturbances due to improper feeding.

To utilize animals infected in the natural way as a source of virus, is to put the experiments into nature's hands as far as possible without losing control of the main conditions. Such experiments cannot, therefore, be completely reproduced at will as is possible in the laboratory with pure cultures, and the results may vary from place to place. They are practical rather than scientific tests in the sense that the practical test may involve unknown factors largely eliminated in the scientific or laboratory test.

A NOTE ON COCCIDIA IN SPARROWS AND THEIR ASSUMED RELATION TO BLACKHEAD IN TURKEYS.

By THEOBALD SMITH, M.D., and ERNEST W. SMILLIE, D.V.M.

(*From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.*)

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The immediate inducements to follow coccidiosis in sparrows were their constant appearance in the unprotected yards of the incubator turkeys described in the preceding article and the view of Hadley[1] that the causal parasite described by one of us in 1894[2] as *Amœba meleagridis* is merely a stage in the development of the avian coccidium *Eimeria avium* and is disseminated and transmitted by various species of birds. Although no proof of this relationship has been furnished and the evidence accumulated is wholly against the hypothesis, one of us has not lost any opportunity to keep it in the foreground for the time being. Coccidiosis is widespread and when the invasion is intense and associated with the blackhead parasite, confusion may arise.

In all, 54 sparrows were trapped or shot while in the turkey yard. No lesions suggesting acute or chronic disease were found in any of them. The search for coccidia was carried on by the examination of fresh scrapings of the duodenal mucosa for the sexual stages and the rectal contents for the oocysts. In addition to this routine examination, cultures were made of the rectal contents of a certain number, to stimulate spore formation, as follows: 2 per cent agar-agar was suspended in a 0.5 per cent aqueous solution of sodium chloride, boiled, cleared, tubed, and autoclaved. When needed, this medium was poured into Petri dishes and allowed to set. Parti-

[1] Cole, L. J., and Hadley, P. B., *Rhode Island Agricultural Experiment Station, Bull. 141*, 1910.

[2] Smith, T., *U. S. Dept. of Agriculture, Bureau of Animal Industry, Bull. 8*, 1895, 7.

cles of feces were smeared over the surface of the agar and the dishes placed in a moist chamber in the incubator at 37°C. and at room temperature.

From a number of sparrows, portions of the duodenum were fixed in Zenker's fluid and alcohol and sectioned for study.

Table I summarizes the observations made.

TABLE I.

Sparrow No.	Sex.	Date.	Duodenum.	Rectal contents.
		1916		
1	Male.	July 12	+++	Not examined.
2	"	" 14	++	" "
3	Female.	Aug. 5	+++	" "
4	?	" 5	+++	" "
5	Male.	" 7	+	" "
6	Female.	" 7	+	" "
7	"	" 9	++	+
8	?	" 10	+	−
9	Female.	" 10	−	++
10	"	" 11	+	+
11	?	" 13	+	−
12	Female.	" 15	+	−
13	Male.	" 15	++	−
14	"	" 15	−	−
15	Female.	" 16	+	−
16	"	" 18	+	−
17	Male.	" 21	++	−
18	Female.	" 23	+++	++
19	"	" 24	+++	+++
20	"	" 28	+++	+
21	"	Sept. 1	++	+
22	Male.	" 3	+	+
23	Female.	" 7	+++	+
24	"	" 8	+++	++
25	"	" 11	+	+
26	"	" 15	+++	+++
27	"	" 15	+++	+++
28	"	" 21	+++	+++
29	"	" 21	+	+
30	Male.	Oct. 1	−	−
31	Female.	" 2	+	+
32	"	" 2	+++	+++
33	"	" 2	+++	+++

TABLE I—*Continued.*

Sparrow No.	Sex.	Date.	Duodenum.	Rectal contents.
		1916		
34	Female.	Oct. 5	−	+
35	"	" 11	++	++
36	"	" 19	+++	+++
37	Male.	" 20	+	+
38	"	" 22	++	+
39	Female.	Nov. 6	+	−
40	Male.	" 9	++	++
41	"	" 9	+	−
42	Female.	" 13	−	−
43	"	" 14	+	−
44	Male.	" 14	+	+
45	"	" 14	−	−
46	"	" 22	−	−
47	"	" 23	+	−
48	Female.	" 23	−	−
49	"	" 23	++	++
50	"	" 28	−	−
51	"	" 28	−	−
52	Male.	Dec. 19	−	−
53	Female.	" 19	−	−
54	"	" 30	−	−

The examinations began on July 12, 1916, and extended to January, 1917. In all, 54 birds were examined and coccidia were found in 43, or 80 per cent. The first thirty-eight sparrows were from the University campus in Princeton, the rest were caught near the Department buildings about 3 miles east of Princeton. The sparrows were all adults weighing from 25 to 35 gm. We knew of no criteria for distinguishing sparrows less than 1 year old from older birds. The relative abundance of coccidia is indicated by the number of plus signs in the columns of the table, the absence of coccidia by the minus sign.

The examination of sections of the small intestine of sparrows, prepared as described, showed an invasion of variable intensity in the duodenum and little if any farther down. The parasites were all in the sexual phase, macrogametes and microgametocytes being the only forms present. The location of all the coccidia was in the

epithelial layer of the villi usually nearer the tip of the villi. The parasites were situated in the nuclear zone of the epithelial cells, close to the basement membrane but not in the core of the villus. Where the villi were twisted or flattened, the gametes appeared to be in the subepithelial tissue, but a careful focussing and consideration of the plane of the section did not confirm such a supposition.

The total absence of the vegetative or multiplicative cycle in the cases studied suggests two possibilities. Either the asexual phase of the cycle is eliminated or suppressed or else it goes on early, possibly while the birds are still in the nest. We have had no opportunity of examining nestlings to verify one or the other supposition.

The cultures of feces were a surprise in as far as they uniformly demonstrated a two-spored oocyst rather than the four-spored species which we had been led to expect from Hadley's observations. Each spore cyst contained four sporozoites. The development of the cyst contents proceeded quite rapidly and was complete within 3 days at room temperature or within 16 hours at 37°C.

It is of interest to note here that in one bird (No. 39) scrapings from the duodenum showed a matured oocyst with two spores, each containing developed sporozoites. This must have been taken in with the food and was detected before the sporozoites had escaped to begin the invasion.

Hadley[3] in a paper on coccidiosis in the English sparrow did not state what the infesting species was, but referred the reader to a later paper. In 1911,[4] he described *Eimeria avium*, a four-spored coccidium, and from the context of the paper the reader must assume that *Eimeria avium* is the sparrow parasite. The two-spored species is not referred to in the above papers and no cultures of the oocysts are mentioned. Some evidence that coccidia infesting turkeys and those infesting sparrows are different is presented by his measurements of the mature cysts which in the turkey average 14 micra by 21 micra, and in the sparrow 22.7 micra by 21.3 micra. The few measurements made by us of cysts from the rectal contents agree closely with his figures of the sparrow coccidium. Thus in two cases

[3] Hadley, P. B., *Centr. Bakteriol., 1te Abt., Orig.*, 1910, lvi, 522.
[4] Hadley, *Arch. Protistenk.*, 1911, xxiii, 7.

the cysts were circular, measuring 18.7 micra and 17.5 micra respectively. In another case the axes were 18.7 micra by 22.1 micra. It is obvious that before drawing generalizations as to the transmission of coccidia from one species of host to another, we must first determine the character of the parasite regularly found in each host. For this determination the measurements of cysts are not very reliable.

The precise nature of the two-spored coccidium in the sparrow we did not attempt to determine. Two-spored coccidia in birds were found by Labbé,[5] who created the genus *Diplospora*. Sjöbring in 1897[6] describes two species in birds, one probably *Eimeria avium* and the other a two-spored species to which he gives the generic name *Isospora*. It is evident that even this superficial dipping into the literature proves the need for a thorough investigation, first of the coccidia of domesticated birds, and second of those of the wild species. It should furthermore be determined which birds are invaded by both species of coccidia and which harbor but one species.

The susceptibility of the incubator turkeys to the coccidia of the sparrows infesting their yards was not conspicuous. As a result of numerous examinations of intestinal scrapings and contents during the whole season, only two turkeys were found harboring coccidia cysts resembling those of the common species.[7] In No. 132, a small number of cysts were found in the cecal and rectal contents measuring 18 micra by 30.4 micra. In feces smeared on agar plates three cysts were subsequently found, two of which had developed each two spores with four sporozoites each. The third cyst remained undeveloped. In a second turkey (No. 155), one cyst was found in the cecum. In neither of these cases were coccidia found in sections of the duodenum, ileum, or ceca. The multiplication must have been very light or the ingested cysts may have passed through undeveloped.

[5] Labbé, A., *Compt. rend. Acad.*, 1893, cxvi, 1300.
[6] Sjöbring, N., *Centr. Bakteriol., 1te Abt.*, 1897, xxii, 675.
[7] Smith, *J. Exp. Med.*, 1917, xxv, 405.

SUMMARY.

Of 54 sparrows examined in or near Princeton, coccidia were found in 43, or 80 per cent. Most of the negative cases were encountered in November and December. In the summer and fall practically all were infected. These figures agree closely with Hadley's, who found 79 per cent infected from May to December.

Cultures of feces on agar showed that, at least in this locality, the infecting species belongs to the genus *Isospora* or *Diplospora* and not to *Eimeria*.

In a recent paper by Hadley,[8] which came into our hands after the manuscript had gone to press, the author now refers blackhead to invasions of *Trichomonas*. It is not possible to consider here the evidence on which this conclusion is based.

[8] Hadley, *Rhode Island Agricultural Experiment Station, Bull. 166*, 1916.

INTESTINAL OBSTRUCTION.

An Experimental Study of the Intoxication in Closed Intestinal Loops.

By LESTER R. DRAGSTEDT, JAMES J. MOORHEAD, M.D., and FRED W. BURCKY.

(*From the Hull Physiological Laboratory of the University of Chicago, Chicago.*)

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The cause of death in acute intestinal obstruction has not been determined. It is generally conceded, however, that a systemic bacterial invasion by the organisms from the obstructed intestine does not occur. As shown by McKenna (1), a bacteremia is only rarely found in clinical cases of death from acute intestinal obstruction. The absence of a systemic bacterial invasion in experimental obstruction has been demonstrated by a number of workers. The theory of a nervous disorder, as a result of anemia of the medullary centers following the loss of fluids in ileus, has received no experimental support. Both the clinical and experimental evidence so far obtained points to a quickly developing and rapidly fatal toxemia.

Hartwell, Hoguet, and Beekman (2) conclude from experiments on dogs that death in experimental intestinal obstruction is due, first, to a dehydration or loss of water from the tissues as a result of the excessive drain into the lumen of the intestine and vomiting, and, second, to the presence of a toxin in the circulating blood. The toxin is believed to be normally present in the lumen of the intestine, and the factor admitting this toxin into the blood is the damage to the intestinal mucosa. The life of the animal after experimental obstruction may be prolonged by the subcutaneous injection of normal saline solution.

Murphy and Brooks (3) conclude from their experiments on dogs with experimental intestinal obstruction, that death is due to toxemia, independent of infection of the peritoneal cavity or the general circulation. The toxin is formed as a result of bacterial growth in the obstructed intestine. It is not absorbed through a normal mucosa, but gains entrance to the blood stream when the mucosa of the intestine has been damaged. They emphasize the significance of the abnormal absorption in the production of the symptoms.

Whipple, Stone, and Bernheim (4) believe that death in these cases of obstruction is due to the absorption directly into the blood of a perverted secretion

of the duodenal or upper jejunal mucosa. They assume a disturbed physiological balance of the mucosa by which abnormal products are formed and secreted into the blood stream. This perverted secretion is a highly toxic substance and they claim that it can be recovered from the lumen of the intestine in cases of obstruction. Some work has been reported on the chemical nature of this toxic substance and attempts have been made to immunize dogs to it.

Draper (5) believes that the cause of death in intestinal obstruction is due to an aberrant activity of the duodenal and probably the pancreatic cells. He appears to have forsaken the view taken by him several years ago of a normal toxic secretion from the duodenal mucosa which is neutralized by the secretions of the intestine lower down.

There has not been a sufficiently thorough investigation of the chemical nature of the toxic substances found in the lumen of the intestine in cases of intestinal obstruction to determine whether they are essentially different from the toxic substances shown by Roger and Garnier (6) to be present in the lumen of the normal stomach and intestine. We should expect to find materials, in such a mixture of food substances and bacterial end-products as exists in the normal alimentary tract, which are toxic when injected into the blood stream. That the substances from the intestine in cases of obstruction are apparently more toxic than the normal contents of the intestine is not surprising. The accumulation of food products and secretions and the unrestricted activity of the intestinal bacteria might easily explain the increase in toxicity. Moreover, an intravenous injection of substances from an obstructed intestine producing toxic symptoms does not indicate that such a procedure occurs in cases of acute obstruction in which these toxic materials are separated from the blood by a layer of living cells. It is the function of the cells to change the substances in the lumen of the intestine to substances which can enter the blood stream without harm to the organism. The normal physiological function of the cells of the intestinal mucosa is both secretory and absorptive. Any pathological alteration (anemia through disturbance to the blood supply, traumatism, etc.) or intoxication of these glandular structures would, we should expect, lead to a depression and not a stimulation of their activity. We should expect a decrease and not a sudden increase in the absorptive properties of these cells under conditions such as exist in intestinal obstruction. Indeed, it has been shown by Braun

and Boruttau (7) and others that there is in reality a decrease in absorption from the intestinal lumen in cases of acute experimental obstruction. If, as maintained by some of the workers, the toxemia of obstruction is due to the absorption of toxic substances normally present in the intestine, made possible through the injury to the intestinal mucosa, this toxemia should be manifest in other inflammatory conditions of the intestine such as are present in ulcer, typhoid fever, amebic dysentery, etc., where there is extensive destruction of the mucosa.

The theory of a non-bacterial or physiological formation of the toxic substances found in the intestine in cases of acute obstruction, as advanced by Whipple and his associates and in a modified form by Draper, is not conclusive. The theory that under conditions of obstruction the normal physiology of the mucosa cells is so changed that they secrete a powerful toxin into the circulation is not probable. Especially is this so since cases of congenital atresia of the duodenum and other parts of the intestine are not rare. The infant shows no signs of toxemia or untoward symptoms until bacteria get into the alimentary tract after birth. The secretions of the intestinal glands begin early in intra-uterine life, and if there is anything significant in obstruction alone in stimulating a toxic secretion, such a secretion and toxemia would be manifest before birth. This does not exclude the factor of bacteria and food coupled with the anatomical condition of obstruction as important in the stimulation to a perverted secretion. From the work of others and from experiments to be reported here, this explanation seems unlikely.

To summarize briefly, death in acute intestinal obstruction is held to be due to a toxemia. That there are toxic substances formed in the intestine is established. That the substances found in the lumen of the intestine in obstruction are more toxic than the substances normally present is indicated. The presence of toxic substances in the blood in obstruction has not been shown. The relation of the toxic substances in the intestine in experimental obstruction to the symptoms, and the factors involved in the production and absorption of these toxic substances are the fundamental questions in the problem of intestinal obstruction which remain unsettled.

All the experiments were done under complete ether anesthesia and with careful aseptic technique. In no case was there an extensive wound infection, and in no instance was there a peritonitis from the operation itself.

Isolated Closed Loops of the First Part of the Jejunum.

Experiments were performed on five dogs, but the results were so uniform that only one protocol will be given.

Dog 1.—May 12, 1916. An isolated closed loop, about 12 cm. in length, of the first part of the jejunum (Text-fig. 1) was made, the continuity of the intestine reestablished by end to end suture, and the ends of the isolated loop were carefully infolded and closed. Care was taken not to injure the blood supply to the isolated loop.

May 13. Dog recovered from the immediate effects of the operation; appeared lively.

May 14. Dog weak; vomited on drinking water.

May 15. Found dead and cold in the cage.

Autopsy.—Performed at once. There was a considerable amount of fluid in the abdominal cavity, and the peritoneum was markedly injected, with evidence of a general peritonitis. The loop was greatly distended and perforated in the center, opposite the mesentery. Aside from the region of the perforation, the mucosa was practically normal. Microscopic examination showed merely a slight round cell proliferation and desquamation of some of the surface cells. The lungs, liver, and kidneys showed no change. There was a slight cloudy swelling in the spleen.

All the dogs in this series died within 96 hours and all showed a perforation of the closed loop and a general peritonitis. The perforation was evidently primarily caused by the accumulation of fluids in the closed loop. The distention was probably sufficient to shut off the blood supply to a portion of the mucosa which, becoming necrotic, rapidly gave way. We believe that death in these cases is due to the perforation and the sudden liberation of bacteria and their toxic end-products into the abdominal cavity where they are rapidly absorbed. The effects of the perforation cannot be essentially different from those in perforated ulcers and there seems to be no reason to believe that the small area of necrosis in these cases plus bacteria could give rise to a sufficient amount of toxic substances rapidly

enough to produce the symptoms observed. The inflammatory condition of the intestine adjacent to the perforation is not more marked than that found in any acute enteritis.

Isolated Closed Loops of the Lower Intestine.

Dog 2.—Apr. 6, 1916. An isolated loop, 12 cm. in length, of the lower part of the jejunum was made, the intestinal canal reestablished by end to end suture, and the ends of the isolated loop were carefully infolded and closed. The blood supply to the loop was not damaged.

Apr. 7. Dog lively.

Apr. 9. Dog appeared toxic; vomited on drinking water.

Apr. 10. Found dead and cold in cage.

Autopsy.—Performed at once. The abdominal organs were normal except for the loop which was markedly distended and perforated causing a general peritonitis.

Dog 3.—Apr. 20, 1916. An isolated loop of the lower end of the ileum about 12 cm. in length was made, the intestinal canal reestablished by end to end suture, and the ends of the isolated loop were carefully infolded and closed. The blood supply to the loop was not damaged.

Apr. 21. Dog lively.

Apr. 25. Dog found dead and cold in cage.

Autopsy.—Performed at once. The closed loop was greatly distended and perforated causing a general peritonitis.

Dog 4.—May 25, 1916. An isolated loop, 12 cm. in length, of the transverse colon was made, the intestinal canal reestablished by end to end suture, and the ends of the isolated loop were carefully infolded and closed. The blood supply to the loop was not damaged.

May 26. Dog lively.

May 28. Dog able to eat and drink; appeared normal.

June 4. Dog died of distemper.

Autopsy.—Perforation of the closed loop which had been sealed by the omentum. There was a localized collection of pus about the perforation, but no general peritonitis.

In two cases isolated closed loops of the intestine in the region of the lower jejunum were made, the dogs living 4 and 5 days, respectively. Death in each instance was caused by a perforation of the closed loop and the liberation of the toxic substances into the abdominal cavity. The dog with the closed loop of the ileum lived 5 days, death being due to the perforation peritonitis. Dogs with closed loops of the colon may live for weeks with no pathological symptoms.

Isolated Closed Loops of the Duodenum.

Dog 5.—July 6, 1916. An isolated loop of the duodenum, about 12 cm. in length, below the lower pancreatic duct was made, the continuity of the duodenum reestablished by end to end suture, and the ends of the isolated loop were carefully infolded and closed, great care being taken not to injure the large duodenal vein which at this point runs parallel to the intestine.

July 7. Dog highly toxic; refused water; lay quietly in corner of cage.

July 8. Dog died 48 hours after the operation.

Autopsy.—Performed at once. No peritonitis. The abdominal organs were normal except the closed loop. The loop was markedly distended and gangrenous but not perforated. The fluid in the loop was of the consistency of thick soup, bloody, and very offensive. It contained great numbers of bacilli and cocci. The mucosa of the loop was necrotic, coming away from the wall of the intestine as a brick red powder.

Six dogs were operated upon as described above and all died within 50 hours. At autopsy there was a perforation of the loop and general peritonitis in two animals, but in the remaining four the loops were distended but intact. In every instance the loop was black and gangrenous as compared with the remainder of the intestine. The mucosa always showed the same change and was markedly necrotic. The lungs, liver, and spleen were practically normal.

Closed Pavlov Pouch.

Dog 6.—Feb. 29, 1916. An operation for making a regular Pavlov pouch was performed, but instead of making a fistula, the small stomach was entirely closed (Text-fig. 2).

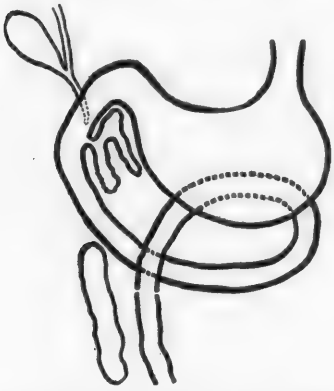
Mar. 1. Dog lively; no signs of toxemia.

Mar. 2. Dog died 54 hours after the operation.

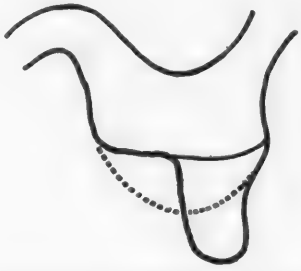
Autopsy.—Performed at once. The small stomach or pouch was greatly distended and perforated opposite the sutures. General peritonitis. The mucosa of the stomach and duodenum was not injected.

The experiments described in the above four groups are in part a duplication of experiments reported by other workers. Our purpose was to corroborate their findings and to establish a basis for our later experiments. They bring out the following facts: (1) Dogs with short closed isolated loops of the intestine die, those with loops in the upper part more rapidly than those with loops lower down. (2) There is no excessive vomiting of fluids with a resultant dehydration

of body tissues. (3) Dogs with closed isolated loops of the jejunum and ileum always show a perforation of the loop and general peritonitis at autopsy. Aside from the perforation the mucosa is not markedly changed. (4) Dogs with closed isolated loops of the duodenum die more quickly than dogs with closed loops in any other part of the intestine. At autopsy there may be an intact closed loop and no signs of peritonitis. In all cases where death occurs rapidly (within 48 hours), the mucosa of the closed loops is necrotic. In these cases the loops are distended with a fluid which contains great numbers of putrefactive bacteria.



TEXT-FIG. 1. Isolated closed loop of the first part of the jejunum.



TEXT-FIG. 2. Closed Pavlov pouch.

Isolated Closed Loops of the Upper Part of the Jejunum, Previously Washed with Sterile Water and Ether.

Isolated loops of the jejunum just below the duodenojejunal flexure were made (Text-fig. 1) and the continuity of the intestine was reestablished by end to end suture. The isolated loops were then carefully washed with about 2 liters of sterile water and then with 2 liters of ether. A spray was used for this work and the fluids were introduced at sufficient pressure to distend the loops, thus affording a thorough washing of the mucosa. Following the washing, the ends of the loop were carefully wiped, infolded, and closed. Twenty-

five dogs were operated on in this manner and the postoperative course varied greatly. Sixteen of the dogs died after an interval varying from 2 to 6 days. In all the sixteen dogs there was a perforation of the loop with a resulting general peritonitis. That there had been a great distention of the closed loop before perforation was shown by the great thinness of the wall in places. The course in these dogs was no different from that in dogs in which the loops were not washed. The remaining nine dogs, which were operated on as above, and in which the isolated loops were first carefully washed with sterile water and ether, quickly recovered from the immediate effects of the operation and never showed any subsequent ill effects. Some of the dogs were later operated on and the loops examined. Others were not interfered with following the operation. They are still alive (5 months) and will probably continue to live indefinitely. Dogs which were opened from 2 to 3 weeks following the operation showed a fairly uniform picture. In nearly all, the ends of the loop remained tightly closed and aside from a slight distention the loops appeared practically normal. The fluid in the loops was gray in color and of the consistency of thick soup. Bacteriological examination showed the presence of *Bacillus coli* and a small coccus in most instances.

Merely washing the isolated loops with sterile water before closure does not suffice to save the animal from death within a week, although it prolongs life somewhat. There are at least four explanations of the absence of symptoms and death in the case of the ether washing of the loop. (1) The specific bacterium causing the elaboration of the toxic product in the closed loop may have been removed. The bactericidal property of the ether itself may have been sufficient to destroy the bacteria, or the hyperemia incited by the ether as an irritant may have been instrumental in aiding phagocytosis. (2) The substrate upon which bacteria act to produce the toxic product may have been washed out by the ether. (3) The secretory activity of the cells may have been sufficiently depressed to prevent the accumulation of enough fluids to distend the intestine to the point of rupture. The recovery of the cells from the effects of the anesthetic was probably sufficiently gradual to permit the establishment of an equilibrium between the amount of secretion into the lumen of the in-

testine and the amount of absorption. (4) The absorptive properties of the cells of the washed loops may have been increased through the removal of the lipoid envelopes of the cells by the ether.

Isolated Closed Loop of the Jejunum, Previously Washed with Sterile Water and Ether.

Dog 7.—June 29, 1916. An isolated loop of the first part of the jejunum about 12 cm. in length was made, carefully washed with 2 liters of sterile water and 2 liters of ether, the ends were infolded and closed, and the continuity of the intestine was reestablished by end to end suture.

June 30. Dog lively.

July 1. No signs of toxemia.

July 4. Dog able to eat and drink; appeared normal.

July 8. Laparotomy and examination of the loop. The ends of the loop remained tightly closed. There was a large perforation 2 by 4 cm. in the middle of the loop opposite the mesentery. No evidence of peritonitis. The peritoneum was smooth and shining. There were a few adhesions about the end to end joint in the intestine. The perforation was closed by double rows of Lembert sutures and the loop dropped back into the abdomen. The blood supply to the returned loop was good.

July 10. Dog weak; lay in corner of the cage.

July 12. Dog well and lively.

July 20. Dog apparently in good condition; etherized.

Autopsy.—The abdominal cavity was normal except for the loop, which was entirely surrounded by omentum. It was tightly closed and moderately distended. The fluid in the loop was thick and yellow in color. The mucosa of the loop was normal on gross and microscopic examination. Cultures taken from the loop fluid remained sterile.

The subsequent perforation of the isolated closed loop, in this experiment, without symptoms of intoxication or evidence of peritonitis indicates that the fluids responsible for the distention of the loop and consequent perforation are not necessarily formed as a result of bacterial activity. They are probably non-toxic secretions from the intestinal cells, the activity of which could not have been permanently inhibited by the ether. The lumen of the intestine must have been practically sterilized by the washings or else the perforation must have occurred before there was any marked proliferation of the bacteria and elaboration of their toxic end-products. In this instance the formation of the closed loop did not suffice so to alter the normal

function of the jejunal cells as to stimulate them to the formation of a toxic secretion, either into the blood stream or into the lumen of the intestine. The perforation of the loop allowed free access of the bactericidal abdominal juices into the lumen of the isolated loop and consequent sterilization, as shown by the negative cultures taken on July 20. This experiment demonstrates that an aseptic isolated closed loop of the upper jejunum is not incompatible with life.

Isolated Closed Aseptic Loop of the Lower Jejunum, in Which the Blood Supply Was Occluded with No Untoward Symptoms.

Dog 8.—Apr. 19, 1916. An isolated closed loop of the lower part of the jejunum about 12 cm. in length was made, having been previously washed with sterile water and ether, the ends were infolded and closed, and the continuity of the intestine was reestablished by end to end suture. The blood supply to the loop was not damaged.

Apr. 21. Dog made a good recovery; no toxic symptoms.

July 3. Dog in good condition; laparotomy and examination of the loop. The isolated loop was open at one end, and the secretions of the loop had free drainage into the abdominal cavity. The loop was normal in appearance. There was no evidence of peritonitis, although a few cheesy white flakes could be found in the mesentery. The end of the loop was closed and the entire blood supply to the loop was occluded by double ligatures around the vessels in the mesentery. Cultures taken of the contents of the loop before closure remained sterile.

July 5. Dog recovered from the operation and showed no toxic symptoms.

Oct. 10. Dog was in good condition and had never shown any ill effects from the operation. Laparotomy and examination of the abdominal contents were done. The intestinal loop had entirely disappeared and the only trace of it that could be found was a bit of fibrous connective tissue in the mesentery in the region of the end to end joint in the intestine.

This experiment confirms the results obtained with Dog 7. The washings probably reduced the bacterial content of the intestine to such an extent that the bactericidal juices of the abdomen were able to cope with the remaining bacteria. It shows that isolated intestinal loops of the lower jejunum can be effectually sterilized by this method and also that isolated closed aseptic loops in the region of the lower jejunum are not incompatible with life. It also establishes the fact that anemic necrosis of the intestine following occlusion of the blood supply produces no toxic symptoms when bacteria are excluded.

There is thus a simple aseptic infarct of the intestine. Evidently then the toxemia of intestinal obstruction cannot be due to circulatory changes in the intestine in the absence of bacteria.

Isolated Closed Loop of the Jejunum. Recovery. Death on Subsequent Occlusion of the Blood Supply.

Dog 9.—June 30, 1916. An isolated loop of the first part of the jejunum about 12 cm. in length was made, washed with sterile water and ether, the ends were infolded and closed, and the continuity of the intestine was reestablished by end to end suture.

July 2. Dog recovered from operation; showed no toxic symptoms.

July 10. Dog in perfect health; laparotomy and examination of the loop. There were no signs of peritonitis. The loop was markedly distended but not perforated. The walls of the loop were normal in appearance, thickness, and color, and the blood supply was good. The blood supply was entirely occluded by double ligatures about the vessels in the mesentery and the loop dropped back into the abdomen.

July 11. 24 hours after the operation the dog was found dead.

Autopsy.—Performed at once. The abdomen was distended with gas and bloody, offensive fluid. The loop was entirely disintegrated and separated from the mesenteric stump. Cultures taken from the abdominal cavity showed *B. coli.*

It will be noted that washing the isolated loops of the intestine does not in itself completely remove all the organisms present. It was the subsequent entrance of the bactericidal abdominal juices in the ruptured loops that completed the sterilization in these cases. *Bacillus coli* and cocci were found in the closed loops that were later examined. In this experiment, then, there were probably a great number of bacteria and their end-products in the lumen of the loop. It has been shown that bacteria proliferate rapidly in these closed loops where the conditions for their growth are favorable. However, the mucosa of the intestine is able to prevent the entrance of bacteria or their toxic products into the blood in sufficient amounts to produce symptoms. This is a normal function of the mucosa of the intestine and is evidently not changed by the condition present in a closed isolated loop. The secretion into the lumen of the loop is practically balanced by the absorption from the intestine so that whatever distension occurs is gradual enough for the blood supply to accommodate itself. Thus a condition of anemia and necrosis of

any part of the loop is prevented. However, when the entire blood supply was suddenly occluded, the loop became at once necrotic, and this necrotic tissue plus the enclosed intestinal bacteria resulted in the formation of toxic products, which when liberated into the abdominal cavity and absorbed caused death. Whether the mere liberation of the contents of the loop without necrosis of the tissue would have caused death we do not know. However, from the fact that the liberation of the contents from a washed loop caused no symptoms, it would seem that this tissue necrosis is an important factor.

Isolated Closed Loops of the Lower Duodenum, Previously Washed with Sterile Water and Ether.

Seven dogs were operated on as in Text-fig. 3, the isolated loop of the lower duodenum was carefully washed with sterile water and ether, and the ends were infolded and closed. All the dogs died within 72 hours. There was no marked difference in the postoperative course of these dogs and the dogs in which the closed loops of the duodenum had not been previously washed. All made an uneventful recovery from the immediate effects of the operation and showed nothing abnormal for the first 12 hours. Then symptoms of toxemia, extreme muscular weakness, vomiting, diarrhea, and muscular tremors, appeared, followed by prostration and death. At autopsy the isolated loops were greatly distended and black or covered with purple blotches. In four animals there was a perforation with signs of a beginning general peritonitis. In three, however, there was no perforation and no signs of peritonitis were present. The loops were all distended and black in appearance. In every instance they were filled with a foul smelling bloody fluid. This fluid was found to contain large numbers of *Bacillus coli*, cocci, and other bacilli. In all the dogs the mucosa was necrotic and in some it appeared as a red granular powder.

There are two probable factors accounting for our failure to keep the dogs with the washed closed loops of the duodenum alive. In the first place the upper part of the intestine is mainly secretory and very slightly absorptive in function. Consequently there is little chance to establish an equilibrium between secretion into the intestine

and absorption from it. Second, the arrangement of the blood supply to the duodenum is different from that in any other part of the digestive tract. The vessels supplying the duodenum run parallel to the lumen of the intestine. Any slight distention of the duodenum may easily occlude these vessels and thus shut off the circulation to the entire duodenum. We attribute the failure to keep dogs with closed loops in this region alive as due to these two factors and not to anything peculiar in the quality of secretion of the glands in the duodenum or in the bacterial flora.

Experiments to Determine the Part of Ether in the Washed Isolated Loops.

Dog 10.—May 17, 1916. An isolated loop of the first part of the jejunum about 12 cm. in length was made, washed with sterile water, the ends were infolded and closed, the continuity of the jejunum was reestablished by end to end suture, and the loop filled with ether and dropped back into the abdominal cavity.

May 19. Dog found dead.

Autopsy.—Performed at once. There was a perforation of the closed loop, and signs of a beginning general peritonitis were present. The mucosa aside from the region of the perforation was normal.

Two dogs were operated on in this manner and both died within 48 hours. The experiment was undertaken to find out if the function of the ether was to remove a substrate upon which bacteria might act to produce a toxic product. If the ether was left in the loop, its germicidal and its anesthetic effects on the cells would be the same, but no substrate would be removed. However, the experiment is subject to the criticism that the ether enclosed in the loop when warmed to the temperature of the body might exert sufficient pressure to rupture the loop and liberate the contents.

Isolated Closed Loops of the Jejunum and Colon, Previously Washed with Sterile Water and 70 Per Cent Alcohol.

Closed loops of the upper part of the jejunum were made in seven dogs, the loops being carefully washed with sterile water and 70 per cent alcohol before closure. Of these seven dogs, five died within 4 days with perforation of the loop and general peritonitis. One died in 5 days with a peritonitis from a leak in the joint. The iso-

lated loop in this animal was distended but intact. Cultures taken from the loop fluid showed *Bacillus coli*, a streptococcus, and *Bacillus subtilis*. One of the dogs lived 18 days, but died with a peritonitis from perforation of the closed loop.

Isolated closed loops of the transverse colon, washed with sterile water and 70 per cent alcohol, were made in three dogs. One dog died after 3 days with a perforation of the loop and resulting general peritonitis. The other two showed no untoward symptoms and were later opened and the loops examined. In one of the dogs opened 19 days after the first operation, the closed loop was found intact but somewhat distended. The other dog was opened after 28 days and the loop removed. Here also the loop was distended and intact. In both these animals the fluid in the closed loops was of a dirty chocolate color, foul odor, and contained great numbers of bacteria.

It will be seen that dogs with alcohol-washed loops live longer than dogs with simple closed loops or dogs in which the loops have been washed with sterile water before closure. It is probable that alcohol acts in much the same manner as ether.

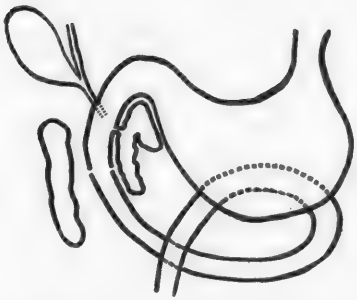
Isolated Loops of the Upper Jejunum, Previously Washed with Sterile Water and Lysol Solution. Ends Closed.

A 2 per cent solution of lysol was used and experiments were performed on two dogs. Both died within 48 hours with a perforation of the closed loop and a general peritonitis. In both the mucosa of the loop was necrotic and separated from the muscularis.

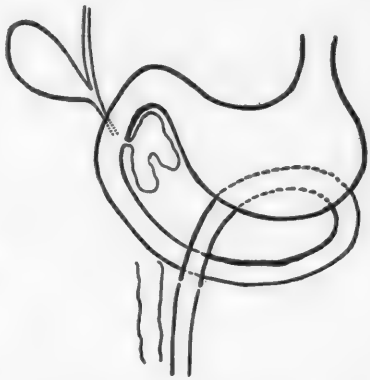
Isolated Loops of the Jejunum, Previously Washed with Sterile Water, Dropped Back into the Abdomen with Both Ends Open.

The perforation of closed loops of the jejunum and the non-appearance of peritonitis or toxic symptoms in some of the previous experiments, led us to attempt experiments of this kind. Four dogs were operated on as in Text-fig. 4, the isolated loops were made in the usual manner but were dropped back into the abdomen with both ends open. All the dogs made uneventful recoveries from the operation and never showed any toxic symptoms. Two of the dogs were

opened 3 weeks after the first operation and the loops examined. The ends remained open although the loops were surrounded by numerous adhesions. There was some light yellow fluid in the abdominal cavity and a few white cheesy flakes in the mesentery. The other two dogs were opened 4 and 5 weeks respectively after the first operation. The loops were buried in adhesions and both ends tightly closed. They were moderately distended. Pressure on the loops



TEXT-FIG. 3. Closed loop of the duodenum.



TEXT-FIG. 4. Open loop of the first part of the jejunum.

caused no escape of fluid from the sealed ends. The fluid in these loops was yellow and quite thick. Cultures on physiological blood agar were negative.

Isolated Loops of the Duodenum Dropped Back into the Abdominal Cavity with Both Ends Open.

Dog 11.—July 20, 1916. An isolated loop of the duodenum about 10 cm. in length below the lower pancreatic duct (Text-fig. 5) was made, the continuity of the duodenum reestablished by end to end suture around the loop, and the loop dropped back into the abdomen without washing and with both ends open.

July 23. Dog recovered from operation; showed no signs of toxemia.

Aug. 10. Dog in perfect health; nutrition has not suffered since operation; laparotomy and examination of the loop. Both ends of the loop were tightly sealed by adhesions. The loop was distended, but pressure applied to it did

not cause any fluid to escape from the ends. It was dropped back and the abdomen closed.

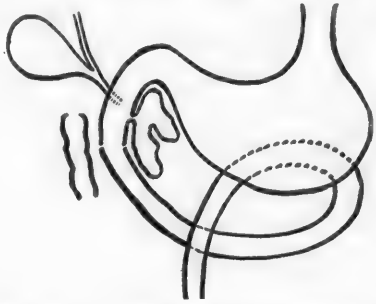
Aug. 11. Dog recovered from the operation.

Aug. 15. Dog apparently in perfect health; no signs of intoxication since operation.

Aug. 20. Dog etherized.

Autopsy.—Performed at once. There was some thin yellow fluid and a few flakes of fibrin were found in the abdominal cavity. The isolated loop was closed and wrapped in dense adhesions. Thick gray fluid was present in the lumen. The mucosa was normal on gross and microscopic examination. No cultures were made of the fluid in the loop.

Six dogs were operated on in the above manner. Three died within 5 days of a general peritonitis. The remaining three showed no



TEXT-FIG. 5. Open loop of the duodenum.

untoward symptoms whatever after the operation. One was opened after 16 days, the isolated loop inspected, and the ends were closed. At operation the loop appeared normal and the blood supply was good. Following the second operation the dog developed a hernia and was etherized 3 days later. The other two dogs were opened 20 and 30 days respectively after the first operation. In both these animals the loops were tightly closed by adhesions and were distended with a thick yellow fluid. Cultures made in one case were negative. In both instances the muscularis and mucosa were normal on gross and microscopic examination.

The experiments with the open loops of the jejunum and duodenum show conclusively that the secretions of the jejunum and duodenum are not toxic enough to induce symptoms in dogs when allowed to

flow into the abdominal cavity. Whether these secretions are toxic when injected into the blood stream, we do not know. All the normal stimuli to the secretion of the glands of the intestinal mucosa are not present, but the secretion goes on, and it is probable that this secretion is qualitatively the same in the isolated open loops as in the intact intestine. There has been no damage to the secreting cells either through injury to the cells themselves directly (trauma in washing) or to their blood supply. The experiments also show that the theory of Draper of a normal toxic secretion of the mucosa of the duodenum which is neutralized by the jejunal juices is untenable. The juices of the open loops of the duodenum are absorbed directly from the abdominal cavity and do not come in contact with the mucosa of the jejunum and ileum. Also there is no perverted secretion or aberrant activity on the part of the cells of the duodenal mucosa when the factor of the bacteria of the intestine is excluded and when the blood supply is not occluded by a too rapid distention of the loops.

DISCUSSION.

It has been demonstrated that death in experimental intestinal obstruction may occur in the absence of a systemic bacterial invasion or of peritonitis. It is probable that the symptoms and death are due to a rapidly developing toxemia, although toxic substances have not been demonstrated in the blood in cases of experimental obstruction. As there was no excessive vomiting of fluids in our experiments the theory of dehydration of the body tissues receives no support. We were able to keep alive animals with closed loops of the duodenum and upper jejunum where bacteria had been previously removed from the loop by free drainage into the abdominal cavity. Inasmuch as the mucosa of these loops was not injured in any way, the theory of a perverted secretion of the mucosa cells induced by the condition of obstruction present in closed duodenal loops, as advocated by Whipple, appears untenable, as is also the theory of Draper of an aberrant activity of the cells of the duodenum and the pancreas. The early theory of Draper that the toxemia in obstruction was due to the absorption of toxic secretions of the duodenum which were normally neutralized by the juices of the intestine lower

down, is disproved by the experiments with the open duodenal and jejunal loops in which these unneutralized secretions pass directly into the abdominal cavity and are absorbed, without the production of toxic symptoms.

We were also able to keep alive dogs with closed loops of the intestine (loops washed with sterile water and ether) containing bacteria, but in which the blood supply had not been damaged by distention. Thus, it is probable that bacteria alone or in connection with the contents of the lumen of the intestine, do not give rise to the toxemia of obstruction, when they are separated from the blood by the cells of the intestinal mucosa. However, a combination of these two factors (necrotic tissue plus putrefactive intestinal bacteria) rapidly gives rise to fatal toxemia. It is probable that the death in the experiments with the unwashed isolated intestinal loops is identical with that following gangrene of the intestine. The fatal toxins are the result of the action of the putrefactive organisms on necrotic tissue. That dogs with closed loops of the duodenum die sooner than dogs with closed loops lower down in the intestine may be due to the fact that the duodenal loop becomes entirely necrotic on distention by reason of the arrangement of the blood supply while in the cases of the loops of the remainder of the intestine only the tissue in the immediate vicinity of the perforation becomes necrotic. Death in the latter cases may be caused by the peritonitis resulting from the perforation. The area of necrosis or gangrene in the lower intestine is usually so small that it is probable that sufficient toxic products to account for the symptoms could not be formed.

Just how far the conditions present in closed isolated unwashed loops of the intestine resemble those found in clinical obstruction is problematic. We believe that the symptoms and pathology of these simple closed loops resemble cases of acute obstruction in man in which there is an accompanying occlusion of the blood supply to a part of the intestine (volvulus, strangulated hernia, etc.). The toxemia resulting from closed intestinal loops is not similar to the toxemia resulting simply from a delay in the passage of food in the intestine and in which there is no disturbance of blood supply or injury to the tissue.

CONCLUSIONS.

1. Closed intestinal loops in which bacteria are first removed are compatible with life.

2. Closed intestinal loops in which bacteria are present but in which tissue necrosis is prevented, are compatible with life.

3. Closed aseptic intestinal loops in which the blood supply is completely occluded are compatible with life.

4. The normal secretions and bacterial products of the duodenum and jejunum are not sufficiently toxic to produce any symptoms when allowed to drain into the abdominal cavity.

5. Our results do not support the theory of Draper of a normal toxic secretion of the duodenal mucosa, neutralized by the jejunal mucosa, or the perverted secretion theory of Whipple.

6. Bacterial activity plus necrotic tissue, or the absorption of toxic products resulting from the action of putrefactive bacteria on necrotic tissue is the important factor in the rapid death in simple closed intestinal loops.

The authors wish to express their thanks to Dr. A. J. Carlson for suggestions and criticism during the course of the experiments and other assistance, and to Dr. C. S. Chase for facilities in the preparation of the paper.

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THE INFLUENCE OF CERTAIN ORGANIC SUBSTANCES ON THE GROWTH OF THE TUBERCLE BACILLUS.

By PAUL A. LEWIS, M.D.

(*From the Henry Phipps Institute of the University of Pennsylvania, Philadelphia.*)

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In attempting to treat experimental tuberculosis by well defined chemical compounds, it is important to know what relations exist between the tubercle bacillus and the chemicals, under various conditions of exposure. The present paper is intended as a contribution to our knowledge of these relations in as far as they can be studied in the culture flask.

HISTORICAL.

The general conception that it might be profitable to determine the power of substances to inhibit the growth of cultures, selecting the more active for subsequent therapeutic experimentation, was formulated by Robert Koch. The idea was completely developed by Bechhold and Ehrlich. The work of Koch and Bechhold and Ehrlich agreed in showing that substances might be highly active in the test-tube and still have no demonstrable capacity to sterilize the diseased animal. Ehrlich, moreover, showed conclusively in the course of his chemotherapeutic studies that substances without activity in the test-tube may be very active against the parasites in the body of the animal. In fact, until recently there was no instance known of a well defined chemical compound having pronounced disinfectant action in the test-tube and exerting the same in demonstrable degree in the animal body.

Schiemann[1] has recently developed the fact that salvarsan is active in the test-tube against anthrax bacilli, the bacillus of swine erysipelas, and *B. mallei* in dilutions of 1 : 1,000,000 or greater. Serum seems not to inhibit this action, but rather to favor it in certain instances. Against experimental infections with these microorganisms the drug also has a certain value. Ethylhydrocuprein (optochin), perhaps the one determinate chemical compound which has up to the present time been markedly effective *in vivo* against a bacterial infec-

[1] Schiemann, O., *Z. Immunitätsforsch., Orig.*, 1915–16, xxiv, 167.

tion (pneumococcus), has marked, and in some degree, at least, specific activities against the tubercle bacillus *in vitro*.

Disinfectants as studied in the test-tube fall into two classes. One class is characterized by the possession of a more or less uniform action against all bacteria. If the action of phenol and mercuric chloride on a variety of bacterial species is compared, it is found that the mercurial compound is by far the more active agent (extraneous albumin being excluded from the reaction). The resistance of bacterial species also varies, some being susceptible, others relatively resistant. But if a ratio is drawn between the activity of phenol and bichloride of mercury for any single species of bacteria, that ratio will be approached closely by similar figures made up on the basis of experiment with any other race or strain of microorganism. Such substances are general disinfectants; they are more or less universal protoplasmic poisons, and, as a corollary to this, are apt to be proportionately far more toxic to higher animals than they are for microparasitic life.

The other, and from the point of view of experimental therapeutics, the more important class of disinfectants, is characterized by a limited range of action. Certain bacterial species only are affected in a marked degree by the typical members of the group. This is the ideal conception and one which is being more nearly fulfilled by the continued examination of substances from this point of view. In no instance, perhaps, up to the present time has the specificity displayed by the bacteriolysins of the blood serum of immune animals been uncovered as an attribute of well defined chemical compounds, although this result must be postulated as ultimately attainable.

The literature of this type of disinfectant action, designated by Ehrlich as *halb specifische*, or partially specific disinfection, is not extensive.

It was shown by Boer (1890)[2] in certain tables that malachite green and methyl violet act differently than a number of other disinfectant substances, showing a somewhat unusual degree of variation in their activity against certain bacteria. *B. anthracis* and *B. diphtheriæ* were about equally resistant to mineral acids,

[2] Boer, O., in Behring's Gesammelte Abhandlungen, Leipsic, 1893, pt. i, 198

the stronger alkaline substances, salts of certain heavy metals, and phenol. *B. anthracis* was the more susceptible to malachite green in the proportion of 12 to 4, and to methyl violet in the ratio of 7 to 1. Boer did not comment on the possible significance of this observation.

Von Drigalski and Conradi (1902)[3] based a method for the differential isolation of *B. typhosus* on what they called the "elective bactericidal action" of certain aniline dyes—malachite green, brilliant green, methylene blue, methyl violet, and Krystallviolett were mentioned by them as showing this property. They recommended the addition to the culture medium of Kristallviolett in certain concentrations, especially to restrain the growth of cocci commonly found in the dejecta. Loeffler (1903)[4] found that a preparation of malachite green containing dextrin, when added to agar plates in concentrations of 1 : 1,000 to 1 : 4,000 restrained the growth of *B. coli* more than that of *B. typhosus*. He worked out and recommended a method for the rapid isolation of the typhoid bacillus on this basis.

Bechhold and Ehrlich[5] and subsequently Bechhold[6] developed most fully the partially specific nature of the disinfectant action of certain substances. The papers of these authors cannot be abstracted with advantage. Concerning our immediate problem, Bechhold reports some observations made with the tubercle bacillus, the substances tested having no demonstrable action against this species. Subsequently in a general review of this work, Bechhold[7] makes the statement that monochloronaphthol is highly active against the tubercle bacillus and *B. pyocyaneus*, but much less active against staphylococcus. He gives no exact figures nor does he state which of the several possible monochloronaphthols he employed.

DeWitt[8] in a study of the power of a number of aniline dyes to restrain the growth of various bacteria, records[9] the development of different bacterial species on agar to which the dyes had been added in the single concentration of about 1 : 8,000 (2 drops of a 1 per cent solution in 10 cc. of agar). Tests at a single concentration are not, of course, designed to bring out any specific quality which substances may possess. DeWitt's tables do, however, give some indication of this action. In most instances *B. subtilis* and *B. tuberculosis* were equally affected. With Bismarck brown, eosin A, erythrosin, and neutral red, the tubercle bacillus gave a feeble growth or none at all, while *B. subtilis* grew vigorously. With eosin (bluish) the reverse was found. In general, the growth of the tubercle bacillus was inhibited more readily than that of any other organism tested.

[3] von Drigalski, and Conradi, H., *Z. Hyg. u. Infectionskrankh.*, 1902, xxxix, 283.

[4] Loeffler, *Deutsch. med. Woch.*, 1903, xxix, 286.

[5] Bechhold, H., and Ehrlich, P., *Z. physiol. Chem.*, 1906, xlvii, 173.

[6] Bechhold, H., *Z. Hyg. u. Infectionskrankh.*, 1909, lxiv, 113.

[7] Bechhold, Desinfektion, in Ehrlich's Festschrift, Jena, 1914, 505.

[8] DeWitt, L. M., *J. Infect. Dis.*, 1913, xii, 68.

[9] See Table V.

DeWitt's results are possibly less significant than they otherwise might be because the culture of the tubercle bacillus which she used was, as she states, of "questionable" authenticity. It was a rapidly growing culture producing abscesses in guinea pigs. She states that: "These abscesses are acute or at least develop much more rapidly than tuberculous glands, and other pigs in the same cage are infected." These characteristics are certainly not typical of the tubercle bacillus.

Our study as presented in this paper represents the beginning of a general survey of available organic chemicals with the view to determining which of them are able in some partially specific sense to restrain the growth of the tubercle bacillus. As will become apparent in the course of the paper, such an effort as this is subject to many limitations both as to method and material and the results can have at most a limited value. They are presented with the hope that they may form a useful guide to others interested in this field of work.

Method.

In general our method has been to make various dilutions in glycerol bouillon of the substances employed. The flasks containing these mixtures were inoculated with a surface growth of the tubercle bacillus, certain control flasks of the same bouillon being inoculated at the same time. When the growth on the control flasks completely covered the surface, the experiment was terminated by a comparison and record of the extent of growth throughout the series. The flasks were then inoculated with one drop of a 24 hour bouillon culture of *Bacillus typhosus* and a similar comparison was made on the following day of the degree of growth of this microorganism. Some comparisons were made with other bacteria to determine the general suitability of the typhoid bacillus as a standard. It would be impossible to cover a wide field of this nature without establishing some sort of routine procedure and we shall describe briefly the one that we have adopted after much preliminary experimentation.

Preparation of Solutions.—The preparation of the test fluids varies with the concentration desired. We use as the basis for our observations 20 cc. of glycerol bouillon (acidity 1.5 to 2 per cent phenolphthalein) in Jena glass boiling flasks of 50 cc. capacity. This gives a comparatively uniform surface area to be covered. For con-

centrations stronger than 1 : 1,000 the substance to be tested is weighed directly into the empty flask. We use a torsion balance for this work. The balance is simple and rapid in its operation and is adjusted to a sensitivity of 1 mg. Weighing 20 mg. and adding 20 cc. of bouillon gives a concentration of 1 : 1,000. The possible error at this concentration is 5 per cent, the probable error is less than this and would be equalized in a series of tests. The flasks are conveniently labeled by a ring of paper cut to hang over the neck.

When a series has been weighed off, the flasks plugged with cotton are sterilized with dry heat. Most of the substances we have studied so far stand a temperature of 130°C. without decomposition when dry. This temperature continued for 3 hours will usually render the preparation sterile. The bouillon measured into similar flasks is separately sterilized in the autoclave. When ready it is poured into the flask with the weighed, sterilized substance. This can be accomplished with only small losses due to contamination. For lesser concentrations, 1 : 1,000 to 1 : 10,000, the 1 : 1,000 solution in bouillon is diluted with glycerol bouillon with suitable pipettes.

For concentration below 1: 10,000 we start with 25 mg. of the substance sterilized by dry heat in a suitable flask and then add sterile water to make a 1 : 1,000 solution. This is then measured into the bouillon flasks; 2 cc. for 1 : 10,000, 1 cc. for 1 : 20,000, etc.

According to the method of preparation, it will be noted that the concentrations given in the tables are only approximately correct. The actual concentrations, 1 : 11,000, 1 : 21,000, 1 : 40,500, etc., can be substituted, but the round numbers are more convenient to handle and are close enough for our present purpose.

Many substances can be more simply handled by sterilizing the finished solution in bouillon. So large a number are either decomposed or enter into fixed combinations with bouillon constituents when treated in this way that we recommend the above procedure as a rule. Certain substances which can be sterilized successfully in watery solution decompose at the temperature mentioned when dry. They are relatively few and must be handled by other procedures.

Cultures Used.—As the culture for all this work we have used a strain of *Bacillus tuberculosis* obtained some years ago from The

Rockefeller Institute for Medical Research designated Bovine III. This culture is probably a transplant of the Bovine III of Theobald Smith. A comparison with the latter strain, however, shows that our culture grows rather more vigorously and is definitely less virulent for guinea pigs. Our culture does, however, give the characteristic disease in guinea pigs and rabbits, is acid-fast, and produces no acid in glycerol bouillon. It is evidently a culture of bovine type of low virulence. The results obtained with this culture have been checked in certain instances with other cultures of higher virulence, also vigorous growers, and found to be identical. We have so far made no comparison with recently isolated cultures, which offer an important problem, but a distinct one.

The stock culture for this work is kept on glycerol bouillon. Cultures about 3 weeks old have usually been used for the inoculation. The inoculation is made with a fine straight wire, a bit of the growth 1 or 2 mm. in diameter being carried over. For each separate lot of culture medium used, at least five control flasks have been inoculated in series with those test substances in which that particular bouillon was concerned. This is essential.

The culture Bovine III can be depended on to give a growth over all of the surface of flasks as we have used them in from 1 to 2 weeks. When the surface of all the control flasks is just overgrown we have terminated the observation period.

For the tests with *Bacillus typhosus* we have used the Rawlings strain obtained some time ago through the courtesy of Major Russell, of the United States Army.

Recording Results.—The results are recorded by an inspection of the flasks. The original bit of culture at least must remain visible if the experiment is to be considered conclusive. If it is shaken down by accident or sinks spontaneously, growth cannot occur from the bottom. The designations used are as follows:

No growth indicates that the original bit is thin and dry; very slight, that the original bit is thickened but shows no new surrounding film; slight, that there is a definite film of new growth extending from the original, the whole mass being 1 cm. or less in diameter; moderate, that the surface is about one-fourth covered; good, that the surface is one-fourth to three-fourths covered; and full, that the surface is completely covered or nearly so.

Occasional lots of bouillon are encountered in which after a period of growth there is a cessation with the surface of the control flasks about three-fourths covered. Tests in which this occurs must be discarded.

The results of the tests with *Bacillus typhosus* are recorded in the same terms based on the examination of a hanging drop taken from the flasks at the end of a 24 hour period of growth. The slight and moderate grade degrees of proliferation are frequently marked by phenomena of agglutination and involution which help to fix the amount of inhibition which prevails. A numerical expression could be obtained by plating, but it is doubtful if this would be of use for the present purpose in view of the fact that no such system could be applied to the tubercle bacillus.

Materials Used.—The substances reported in this paper are nearly all aniline dyes. They have been obtained partly by purchase in the market and partly by donation.[10] These commercial dyes contain a varying proportion of impurities, especially inorganic salts. They should in many instances undoubtedly be considered as mixtures of more or less closely related substances rather than as chemical entities. The salts should be considered as diluents. In the concentrations we are considering they probably have no effect on growth. In so far as it is possible that other impurities may exist in any preparation and increase its activity, the results now reported cannot be considered as final. On the whole, this is probably not a factor in many instances. To exclude it as a possibility in any case involves so much careful work that it can only be attempted on a selected material and as a special problem. We have already approached this matter in certain cases which seemed to be especially significant in their bearing on the influence of chemical constitution on the result. Such observations as we have made will be reserved for later publication.

[10] We are indebted to the American agents of Kalle and Company, and the Bayer Company, for sample lines of the azo-dyes put in the trade by their respective firms. We are also indebted to Professor W. T. Taggart of the University of Pennsylvania for large contributions from a collection of dyes maintained by the Department of Organic Chemistry. The dyes purchased have been obtained chiefly from Grübler and Kahlbaum.

RESULTS.

The results of this survey as carried out up to the present time are given in Tables I to VI. The tables show the name of the preparation tested and its source. They show the greatest concentration at which growth is full for each culture of *Bacillus tuberculosis* and *Bacillus typhosus*. These concentrations are combined in a factor which is an expression of the relative susceptibility to the inhibiting action of the dye. The point of full growth was taken as the basis of this comparison because it permits of the consideration of a much larger number of substances than does the point of complete inhibition. The point of no growth for the typhoid bacillus can be determined in comparatively few instances. In certain cases it has been possible to compare the influence of substan ce for all concentrations from no growth to full growth with each bacterial species. In these cases the factor has been much the same with whichever concentration was made the basis for comparison.

The substances have been arranged in the tables in groups according to their activity against the tubercle bacillus. With the substances showing much activity it is usually possible to show a restraining action on the typhoid bacillus at some concentration. For less active substances the typhoid organism frequently grows without restraint in a saturated solution. In these cases it is only possible to indicate that the factor is greater than the value actually determinable.

The interest of the laboratory has centered for some time on the action of certain azo-dyes which are vital stains. For this reason the number of this class of dyes examined is large, and out of proportion to those of other constitution. These are tabulated by themselves in the case of the more active ones. In making this separation we have relied in most instances on the tables of Schultz.[11]

Interpretation of Results.—There is perhaps little that can be said at this time in extension of the actual results of the work presented. It is evident that a great variety of substances have marked capacity to restrain the growth of the tubercle bacillus. Those dyes whose disinfectant activities have long been known to be considerable,

[11] Schultz, G., Farbstofftabellen, Berlin, 5th edition, 1914.

TABLE I.

Dyes of Various Groups Strongly Restraining Growth of the Tubercle Bacillus.

Designation.	Source.	Tubercle bacillus growth full at.	Typhoid bacillus growth full at.	Typhoid bacillus / Tubercle bacillus
Acridinorange	G.*	1 : 1,000,000	1 : 4,000	$\frac{250}{1}$
Äthylrot	Kahl.	1 : 200,000	1 : 1,000 (Saturated.)	$\frac{200}{1}+$
Auramin	G.	1 : 200,000	1 : 4,000	$\frac{50}{1}$
" O	Kahl.	1 : 200,000	1 : 4,000	$\frac{50}{1}$
Aurantia (Kryst.)	G.	1 : 4,000,000	1 : 1,000 (Saturated.)	$\frac{4000}{1}+$
Azur II	"	1 : 200,000	1 : 2,000	$\frac{100}{1}$
Chinolinrot	Kahl.	1 : 400,000	1 : 40,000	$\frac{10}{1}$
Chrysanilin	"	1 : 200,000	1 : 4,000	$\frac{50}{1}$
Coerulein S.	G.	1 : 100,000	1 : 1,000 (Saturated.)	$\frac{100}{1}+$
Cresylechtviolett	"	1 : 400,000	1 : 2,000	$\frac{200}{1}$
Krystallviolett	"	1 : 1,000,000	1 : 100,000	$\frac{10}{1}$
" (Hoechst)	"	1 : 200,000	1 : 100,000	$\frac{2}{1}$
Dahlia	"	1 : 100,000	1 : 10,000	$\frac{10}{1}$
Diamantfuchsin	"	1 : 100,000	1 : 4,000	$\frac{25}{1}$
Heliotrop	Kalle.	1 : 4,000,000	1 : 8,000	$\frac{500}{1}$
Indaminblau N extra	Kahl.	1 : 200,000	1 : 2,000	$\frac{100}{1}$
Janusgrün	G.	1 : 400,000	1 : 8,000	$\frac{50}{1}$
Magentarot	"	1 : 200,000	1 : 5,000	$\frac{40}{1}$
Mauvien	"	1 : 400,000	1 : 1,000 (Saturated.)	$\frac{400}{1}+$
Methylene blue	Merck.	1 : 1,000,000	1 : 2,000	$\frac{500}{1}$

* In the tables, G. indicates Grübler; Kahl., Kahlbaum; Kalle, Kalle and Company; By., Bayer and Company. The other names are self-explanatory.

TABLE I—*Concluded.*

Designation.	Source.	Tubercle bacillus growth full at.	Typhoid bacillus growth full at.	Typhoid bacillus / Tubercle bacillus
Methylengrün	G.	1 : 200,000	1 : 2,000	$\frac{100}{1}$
Methylenviolett	"	1 : 2,000,000	1 : 4,000	$\frac{500}{1}$
Methylviolett (Kryst.)	Kahl.	1 : 200,000	1 : 100,000	$\frac{2}{1}$
" B	G.	1 : 200,000	1 : 20,000	$\frac{10}{1}$
" 5B	"	1 : 200,000	1 : 40,000	$\frac{5}{1}$
" 6B extra	Kahl.	1 : 400,000	1 : 40,000	$\frac{10}{1}$
Neutral blue W	Kalle.	1 : 200,000	1 : 1,000 (Saturated.)	$\frac{200}{1}+$
" red	G.	1 : 400,000	1 : 100 (Incomplete.)	$\frac{4000}{1}+$
Nilblau (sulfat)	"	1 : 2,000,000	1 : 4,000	$\frac{500}{1}$
Orcein	"	1 : 100,000	1 : 800	$\frac{125}{1}$
Orcin	"	1 : 100,000	1 : 2,000	$\frac{50}{1}$
Phosphin I	Unknown.	1 : 400,000	1 : 2,000 (Saturated.)	$\frac{200}{1}+$
" 3R	Kahl.	1 : 400,000	1 : 8,000	$\frac{50}{1}$
Resorcin fuchsin	G.	1 : 100,000	1 : 2,000	$\frac{50}{1}$
Rosanilin-acetat	Kahl.	1 : 200,000	1 : 4,000	$\frac{50}{1}$
Safranin 3B	Kalle.	1 : 2,000,000	1 : 4,000	$\frac{500}{1}$
" base	Unknown.	1 : 1,000,000	1 : 8,000	$\frac{125}{1}$
" MN	Badische.	1 : 2,000,000	1 : 4,000	$\frac{500}{1}$
" rein	G.	1 : 400,000	1 : 8,000	$\frac{50}{1}$
Spiller's purple	"	1 : 200,000	1 : 200,000	$\frac{1}{1}$

TABLE II.

Azo-Dyes Strongly Restraining Growth of the Tubercle Bacillus.

Designation.	Source.	Tubercle bacillus growth full at.	Typhoid bacillus growth full at.	Typhoid bacillus / Tubercle bacillus
Alkaligrün	G.	1 : 200,000	1 : 200 (Saturated.)	$\frac{1000}{1}+$
Anilingelb	"	1 : 100,000	1 : 4,000	$\frac{25}{1}$
Benzobraun G	By.	1 : 200,000	1 : 200 (Saturated.)	$\frac{1000}{1}+$
Biebricher Scharlach	G.	1 : 200,000	1 : 100 "	$\frac{2000}{1}+$
Chromanilbraun R	Kahl.	1 : 100,000	1 : 100 "	$\frac{1000}{1}+$
Chrysamin	G.	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Chrysoidin	"	1 : 100,000	1 : 2,000	$\frac{50}{1}$
" extra	Kahl.	1 : 100,000	1 : 2,000	$\frac{50}{1}$
Cloth red 3B extra	By.	1 : 200,000	1 : 1,000 (Saturated.)	$\frac{200}{1}+$
" " 3G "	"	1 : 200,000	1 : 1,000 "	$\frac{200}{1}+$
Dicyanin	Kahl.	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Doppelponceau 4R	By.	1 : 100,000	1 : 4,000 "	$\frac{25}{1}+$
Goldorange	G.	1 : 200,000	1 : 1,000 "	$\frac{200}{1}+$
Havannabraun I	Kahl.	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Helianthin	G.	1 : 200,000	1 : 1,000 "	$\frac{200}{1}+$
Indian yellow G	By.	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Mandarin G extra	Kahl.	1 : 200,000	1 : 200 "	$\frac{1000}{1}+$
Metanilgelb 1a	G.	1 : 400,000	1 : 1,000 "	$\frac{400}{1}+$
" extra	Kahl.	1 : 400,000	1 : 1,000 "	$\frac{400}{1}+$

TABLE II—*Concluded.*

Designation.	Source.	Tubercle bacillus growth full at.	Typhoid bacillus growth full at.	Tubercle bacillus / Typhoid bacillus
Methylorange.....	G.	1 : 200,000	1 : 1,000 (Saturated.)	$\frac{200}{1}+$
Naphtaminbraun 2B.....	Kalle.	1 : 200,000	1 : 200 "	$\frac{1000}{1}+$
" GX	"	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Naphtamin fast black SDE.....	"	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Naphtamingrün A.....	"	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
" AG	"	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
" B.....	"	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Naphtaminviolett N.....	"	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Naphthylamine black NR ..	"	1 : 100,000	1 : 200 "	$\frac{500}{1}+$
Orange IV.....	Kahl.	1 : 1,000,000	1 : 1,000 "	$\frac{1000}{1}+$
Pyraminorange RT.....	Badische.	1 : 100,000	1 : 100 (Incomplete.)	$\frac{1000}{1}+$
Säurebraun.....	G.	1 : 100,000	1 : 100 (Saturated.)	$\frac{1000}{1}+$
Tropaeolin OO.....	"	1 : 100,000	1 : 1,000 "	$\frac{100}{1}+$
Wollschwarz.....	"	1 : 400,000	1 : 400	$\frac{1000}{1}$

the methyl violets for example, are no more active than many others whose general action is limited, as represented by the *Bacillus typhosus* results. The power to inhibit the growth of the tubercle bacillus is limited to no general class of coal-tar dye. It is noteworthy that a great many azo-dyes possess this characteristic although few of them are active against *Bacillus typhosus* in a marked degree.

A general view of the results suggests that in the tubercle bacillus we have a bacterium which is highly sensitive to slight modifica-

TABLE III.

Dyes of Various Groups Restraining Growth of the Tubercle Bacillus Moderately.

Designation.	Source.	Tubercle bacillus growth full at.	Typhoid bacillus growth full at.	Tubercle bacillus / Typhoid bacillus
Anthracenblau.....	G.	1 : 40,000	1 : 1,000 (Saturated.)	$\frac{40}{1}+$
Brillantkresylblau.....	"	1 : 40,000	1 : 1,000	$\frac{40}{1}$
Curcumein N.....	"	1 : 40,000	1 : 1,000 (Saturated.)	$\frac{40}{1}+$
Echtrot.....	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Indulin.....	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Isaminblau 8B.....	Cassella Color Co.	1 : 20,000	1 : 100 "	$\frac{200}{1}+$
Kresofuchsin.....	G.	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Malachitgrün Ia.....	"	1 : 20,000	1 : 4,000	$\frac{5}{1}$
Muscarin.....	"	1 : 40,000	1 : 1,000 (Saturated.)	$\frac{40}{1}+$
Nachtblau.....	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Naphtalinrot.....	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Pararosanilin chlorhydrat...	Kahl.	1 : 40,000	1 : 2,000	$\frac{20}{1}$
Pyronin.....	G.	1 : 20,000	1 : 4,000	$\frac{5}{1}$
Rhodamin B.....	Kahl.	1 : 20,000	1 : 100 (Incomplete.)	$\frac{200}{1}+$
Victoriablau B.....	"	1 : 20,000	1 : 10,000	$\frac{2}{1}$
" 4R.....	G.	1 : 20,000	1 : 10,000	$\frac{2}{1}$

TABLE IV.

Azo-Dyes Restraining Growth of the Tubercle Bacillus Moderately.

Designation.	Source.	Tubercle bacillus growth full at.	Typhoid bacillus growth full at.	Tubercle bacillus / Typhoid bacillus
Bordeaux BX.....	By.	1 : 20,000	1 : 100 (Saturated.)	$\frac{200}{1}+$
Chrysamin G.....	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Diamantschwarz PV.....	"	1 : 20,000	1 : 200 "	$\frac{100}{1}+$
Doppelponceau 2R.....	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Naphtaminschwarz 2RE....	Kalle.	1 : 20,000	1 : 100 "	$\frac{200}{1}+$
" 3RE....	"	1 : 20,000	1 : 2,000	$\frac{10}{1}$
Naphtaminblau 12B.....	"	1 : 20,000	1 : 100 (Saturated.)	$\frac{200}{1}+$
" BE.....	"	1 : 20,000	1 : 100 "	$\frac{200}{1}+$
" BXG.....	"	1 : 20,000	1 : 100 "	$\frac{200}{1}+$
" BXR.....	"	1 : 20,000	1 : 100 "	$\frac{200}{1}+$
Naphtaminbraun H.....	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
" RB.....	"	1 : 40,000	1 : 400 "	$\frac{100}{1}+$
Naphtamindirektschwarz C.	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Naphtamindirektschwarz RWK.....	"	1 : 20,000	1 : 400 "	$\frac{50}{1}+$
Naphtaminechtscharlach B..	"	1 : 40,000	1 : 1,000 "	$\frac{40}{1}+$
" R.	"	1 : 40,000	1 : 1,000 "	$\frac{40}{1}+$
Naphtaminorange 2R.....	"	1 : 40,000	1 : 1,000 "	$\frac{40}{1}+$
Naphtaminrot H.....	"	1 : 40,000	1 : 1,000 "	$\frac{40}{1}+$
Naphtylamingelb.....	G.	1 : 40,000	1 : 4,000	$\frac{10}{1}$

TABLE IV—*Concluded.*

Designation.	Source.	Tubercle bacillus growth full at.	Typhoid bacillus growth full at.	Tubercle bacillus / Typhoid bacillus
Neucoccin.....	Kahl.	1 : 20,000	1 : 100 (Incomplete.)	$\frac{200}{1}+$
Purpurin sicc. opt.....	G.	1 : 20,000	1 : 1,000 (Saturated.)	$\frac{20}{1}+$
Roseazurin G.	By.	1 : 20,000	1 : 4,000 "	$\frac{5}{1}+$
Sulfonazurin D.....	"	1 : 20,000	1 : 1,000 "	$\frac{20}{1}+$
Sulfoncyanin G.....	"	1 : 40,000	1 : 1,000 "	$\frac{40}{1}+$
Wollschwarz 4B.....	Kahl.	1 : 20,000	1 : 200 "	$\frac{100}{1}+$

tions of growth conditions and that we are dealing with a reaction here which bears no real relation to the more usual forms of disinfectant action. This may be the case, but the explanation is hardly constructive. We hope to have for presentation in the near future a series of tests with the pneumococcus carried out on similar lines. It is apparent from what we have done with the pneumococcus that sensitiveness to reaction and exact constitution of the culture medium does not imply sensitiveness to the inhibiting action of dyes as we have developed it in the case of the tubercle bacillus.

Many of these dyes, in fact by far the larger number, stain the growing membrane with greater or less intensity. The individual bacilli in the membrane are much less frequently stained and then only by concentrations much greater than those required to inhibit growth. The restraint of growth seems in no way related to the staining of the membrane.

The staining of the membrane may in some instances possibly render the figures inaccurate. In the case of dyes exhibiting great activity, such as heliotrope, methylene blue, and others, the membrane absorbs the dye from weak solutions so that the concentration in the immediate vicinity of the bacilli is much higher than the dilution number would imply. Whether this increased concentration

TABLE V.

*Weaker Dyes in Concentrations of 1 : 1,000 to 1 : 10,000 Showing Full Growth of the Tubercle Bacillus.**

Designation.	Source.	Designation.	Source.
Acridinrot	G.	Naphtamindirektschwarz EK	Kalle.
Alizarin red	Eimer and Amend.	Naphtamindirektschwarz O	"
Alizarincyanin	Kahl.	Naphtamindirektbraun 2R	"
Alkaliblau 3B	"	" GR	"
Anilinblau	G.	" V	"
Aniline blue black	"	Naphtamine fast black KS	"
Azosäureblau 2G	By.	Naphtamingrün AB	"
Azoblau	G.	" TE	"
Bayerischblau	Kahl.	Naphtaminorange FB	"
Benzoolive	By.	Naphtaminscharlach B	"
Blauschwarz	G.	Naphtaminviolett BE	"
Bordeaux G	By.	Naphtholgelb S	G.
Brillantcrocein 3BA	"	Naphtholgrün B	"
Chromgelb D	"	Naphtholorange	Kahl.
Congo-corinth	G.	Naphtholschwarz B	"
Congo red	Unknown.	Naphthylaminschwarz BN	Kalle.
Congorubin	Kalle.	" BNN	"
Echtgrün	G.	" BOO	"
Heliotrop B	Kalle.	" NA	"
Isaminblau 6B	Cassella Color Co.	Nigrosin	G.
" R	Cassella Color Co.	Patentblau A	Kahl.
Jodgrün	G.	Phloxinrot	G.
Lichtgrün	"	Ponceau PR	"
Magdalarot echt	"	" (Kryst.) 6 RF	Kahl.
Methylgrün rein OO	"	Resedagrün	"
Naphtaminschwarz GE	Kalle.	Rosazurin B	G.
" H	"	Rose bengale	"
" HRE	"	Säuregrün	"
" 4RE	"	Säureviolett 6B	Kahl.
Naphtaminblau 2BK	"	" (Kühne)	G.
" 3RE	"	Sulfonsäureblau R	By.
Naphtaminbrillantblau BW	"	Sulforhodamin	Metz and Co.
" G	"	Thiazinbraun	G.
Naphtaminbraun 8B	"	Tropaeolin	Kahl.
" 4G extra	"	Trypanrot	G.
		Victoriascharlach 4R	Kahl.
		Violettschwarz	G.

* The $\frac{\text{tubercle bacillus}}{\text{typhoid bacillus}}$ factor is indeterminable; except in a few instances, because of the inactivity of the dyes against the typhoid bacillus.

TABLE VI.

Very Inactive. Full Growth at 1 : 1,000 or Stronger.

Designation.	Source.	Designation.	Source.
Äthylgrün (Kryst.)	Kahl.	Fast brown 26048	By.
Aniline red	Merck.	" yellow extra	"
Azocarmin	G.	Flavazin G	Kahl.
Azoeosin	By.	Fluoresceïn	G.
Azogrenadin L	"	Fuchsin S	"
Azolitmin	A. H. Thomas and Co.	Gallein (sicc.)	"
		Gentianablau 6B	Kahl.
Azorubin S	Kahl.	Isaminblau B	Cassella
Baumwollblau	"		Color Co
Benzoazurin	G.	Methyleosin	G.
" G	Kalle.	Naphtaminschwarz RGE	Kalle.
Benzopurpurin	G.	Naphtaminblau 7B	"
Benzo sky blue	By.	" 2BL	"
Bittermandelölgrün	G.	" JE	"
Bleu de Lyon	"	Naphtaminbrillantblau B	"
Bordeaux R	Kahl.	" BWO	"
Brillantazurin 5G	By.	Naphtamintiefschwarz HW	"
Brillantgrün	Kahl.	Naphtaminechtschwarz SE	"
"	G.	" TE	"
Brillantschwarz	"	Naphthylaminschwarz 10B	By.
Carminic acid Ia	"	Orange G	G.
Chinablau	"	Oxaminviolett	Badische.
Chinagrün	"	Patentsäure rubin	G.
Chinolingelb (wasserlöslich)	Kahl.	Ponceau 3R	Kahl.
Chinolingelb	Uncertain.	" 4R	"
"	Badische.	Rapid filter gelb	"
Chloraminorange G	By.	" " grün I	"
Chromotrop	G.	" " rot I	"
" 2R	Kahl.	Rosazurin G	G.
Crocein	G.	Rubin S	"
Diaminblau 3B	Kahl.	Säurecyanin G	Kahl.
" B	"	Säureschwarz B	"
Direktblauschwarz 2B	By.	Säureviolett 1897	G.
Direktgelb R	"	Tartrazin	By.
Echtrot D	Kahl.	Thiazinrot	G.
Eosin (bläulich)	"	Thiazolgelb	Kahl.
" (clairl.)	G.	Victoria violet 4BS extra	By.
" (gelblich)	Kahl.	Wasserblau	G.
" (rein franz.)	G.	" 3B	Kahl.
Erythrosin	"	" 6B	"

is effectively applied against the growth of the microorganism cannot be determined.

The figures presented above, studied in conjunction with what is known of the chemical constitution of the dyes, are suggestive. The quality of the material does not permit accurate judgment and as we expect to have ready shortly similar observations made with especially prepared or purified substances much more suited for these considerations, this discussion will be deferred for the present.

An important question raised by these observations is the relation between the capacity to restrain growth as shown above, and true disinfectant action or killing power of the substances in question. It has been repeatedly shown that in the case of true disinfectants their capacity to restrain growth is roughly proportional to their killing power. In the tables it appears that the dyes which have long been recognized to have disinfectant action, certain of the triphenyl methanes particularly, belong to the group of substances with a high power to restrain the growth of the tubercle bacillus. In all previous consideration of this question, the starting point has been the substance whose lethal action was known. That there might be substances having marked capacity to restrain growth with no corresponding capacity to kill does not seem to have occurred to previous investigators in this field.[12] Contrasting the great inhibitory power of methylene blue in our hands and the low killing power of this dye in the work of DeWitt with the previous observations on this relation, we may safely conclude that there is no close or constant correlation between the two forms of action. Special work directed towards this point is required before a precise conclusion can be reached.

SUMMARY.

The power of a large number of aniline dyes to restrain the growth of *Bacillus tuberculosis* and *Bacillus typhosus* has been determined. Many substances have been found with especial restraining power

[12] Gotschlich, E., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, iii, 479, states that formaldehyde has, as contrasted with other disinfectants of similar lethal value, a high capacity to inhibit growth.

for the tubercle bacillus under the conditions of the test. This capacity to restrain growth in the case of the tubercle bacillus apparently bears no simple relation to true disinfectant action. Opinion as to whether the active substances exert a truly specific activity against the tubercle bacillus or whether the activity is determined by the peculiar conditions imposed by the growth of this bacterium as a surface membrane are left for future consideration.

PROTEOSE INTOXICATIONS AND INJURY OF BODY PROTEIN.

I. The Metabolism of Fasting Dogs Following Proteose Injections.

By G. H. WHIPPLE, M.D., and J. V. COOKE, M.D.

(From the George Williams Hooper Foundation for Medical Research, and the Department of Pathology of the University of California Medical School, San Francisco.)

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In recently reported experiments (1), we have pointed out that the non-protein nitrogen of the blood may show a considerable increase above normal due to intestinal obstruction or the presence of a closed intestinal loop. A definite rise in blood non-protein nitrogen may be found in association with general peritonitis or septicemia, and an acute rise can be demonstrated following an injection of a toxic proteose (1). We have repeatedly observed an increase of over 100 per cent non-protein nitrogen in the blood within 3 or 4 hours following an intravenous injection of 100 to 300 mg. of purified proteose obtained from closed intestinal loops. Such an increase can scarcely be accounted for by any kidney retention of non-protein nitrogen. This observation suggested strongly that there might be a marked protein or tissue disintegration associated with proteose intoxications.

The experiments reported below give a suitable explanation for the high non-protein blood nitrogen in the acute intoxications. They show that the injection of a toxic proteose will cause a profound upset in the body metabolism. Fasting dogs which have reached a base-line of nitrogen elimination when given a single proteose injection will show a remarkable increase in total urinary nitrogen—often an increase of 4 to 6 gm. of nitrogen above the base-line level. This indicates a considerable destruction of body protein,

which is effected by the poison. With a distinct proteose intoxication, there are evident clinical signs and symptoms, vomiting, diarrhea, low blood pressure, and a temperature reaction. Curiously enough the greatest amount of nitrogen is usually excreted during the second 24 hours following the injection when the dog may appear in approximately a normal condition. Evidently the tissue disintegration produced by the proteose is not so prompt as the clinical reaction or there is considerable delay in elimination of the end-products which result from this tissue injury. The reaction often lasts 4 to 6 days after a single injection, and indicates a profound disturbance of protein metabolism.

Methods.

All the experiments were performed upon fasting dogs. The tables show the urinary and fecal nitrogen during fasting periods. The dogs were kept in standard metabolism cages with wire mesh floors and metal bottoms having a sharp pitch toward the collecting spout. This type of cage assured the minimum amount of urine evaporation. The urine flowed into a glass container in which were placed 2 or 3 cc. of glacial acetic acid to neutralize any chance alkaline reaction of the urine. At 10 a.m. each day the dogs were catheterized, and the bladder was rinsed out and left empty. The cages were then cleaned, and the cage washings carefully saved to be used in making up the urine to a unit volume of 1 or 2 liters. If solid feces were passed, the material was kept separate from the cage washings, but semifluid or fluid feces were combined with the cage washings and analyzed as a unit. The total nitrogen of urine and feces was determined in the usual way by the Kjeldahl method. All determinations were done in duplicate.

It will be noticed that the dogs were not given uniform amounts of water each day, as is desirable to obtain a uniform basal nitrogen elimination during periods of fasting. There were two reasons for this. Dogs with proteose intoxications of various kinds are apt to vomit, and will vomit very promptly any fluid given by stomach, which tends to complicate the picture. Secondly, it is not necessary to have these dogs on a uniform base-line of nitrogen elimination, because the reactions following the proteose injections are so marked. The dogs were permitted to drink water as they desired, but no food of any kind was given during the experimental period, unless a note is made to that effect.

Proteose injections were usually given about noon or shortly after that time. In all the experiments the dogs were lightly anesthetized for about 5 minutes to facilitate the injection of the proteose solution into the jugular vein. Injections were made rather slowly by means of a needle in the vein, but were usually completed during an interval of 3 minutes.

The proteose was obtained from human or dog material, as indicated in the various tables. The preparation and isolation of such proteose material have been described in another publication (2). The fresh material from a case of obstruction or a closed intestinal loop may in some cases be diluted with distilled water, if it is too thick to handle easily, and then precipitated with five volumes of 95 per cent alcohol. This precipitation is complete in a few hours, and the precipitate can be used then or after a period of weeks, as no change occurs in this alcoholic mixture. The precipitate is collected by centrifugalization and dissolved in distilled water usually to a volume three times greater than the volume of fluid originally precipitated by the alcohol. This solution may be rich in albumins. It is brought to a boil, and a few drops of acetic acid are added to promote coagulation, a reaction faintly acid to litmus. The coagulum is removed by filtration, leaving a clear, slightly opalescent fluid. This is made faintly alkaline to litmus, brought to a boil, and cooled. This may give a faint precipitate, which is removed by filtration. The solution contains a toxic proteose, and may be given as such intravenously. If desired, this solution may be again precipitated by alcohol, collected in the centrifuge, dissolved in water, boiled in a faintly acid solution, filtered, and made up to faint alkalinity. Or further purification can be accomplished by one-half saturation with magnesium sulfate, removal of the precipitate, solution in water, and dialysis to remove the excess of salt. The toxic fraction in this material is easily precipitated by alcohol or magnesium sulfate, and the chemical reactions as well as its biological characteristics indicate that this substance is a primary proteose (2).

EXPERIMENTAL OBSERVATIONS.

Dog 16–110.—(Table I.) Sturdy, black cocker spaniel; adult male. February 11, 1916. Isolation in metabolism cage with no food. The dog was normal, and no injections of proteose had been given previously.

The proteose was obtained from a human case of intestinal obstruction at operation. This fresh material was precipitated with alcohol, and the proteose isolated in the usual manner. The same material was used in both injections (Table I), in the first injection 25 cc., and in the second injection 35 cc. of a standard solution. The clinical reaction is much the same in the two experiments, showing some increased tolerance for the poison. The increase in nitrogen elimination lasts longer following the first injection, but the total increase above the mean is about the same in both instances, about 6 gm. of nitrogen.

Dog 16–109 (Table II) gives a better illustration of the tolerance to

proteose injection which may develop promptly following the initial injection and protect the body against the injury which is evidenced by clinical symptoms and the rise in the curve of urinary nitrogen.

Dog 16–110 also shows a distinct diuresis following the proteose injection. The volume output of urine was not followed carefully in this experiment, but in a later experiment we find that this dog put

TABLE I.

Proteose Injections. Total Nitrogen Elimination.

Dog 16–110. Normal.

Day.	Urine.		Feces nitrogen.	Weight.	Remarks.
	Nitrogen.	Amount.			
	gm.	*cc.*	*gm.*	*lbs.*	
1	2.91	—	0	29.0	Fasting previous 4 days.
2	3.02	—	0.62*	28.5	
2	Proteose from human intestinal obstruction. Moderate intoxication.				
3	2.06	—	1.09*	27.5	Feces and vomitus. Temperature 38.5°C.
4	5.03	—	0	27.3	Temperature 38.4°C.
5	4.42	800	0	26.8	Diuresis. Dog normal.
6	4.64	550	0	26.5	
7	3.60	550	0	26.1	
8	2.97	—	0	26.1	Temperature 38.3°C.
8	Proteose from human intestinal obstruction. Larger dose and same reaction.				
9	3.64*	—	—	25.5	Definite intoxication.
10	5.82	1,100	0	24.9	Diuresis. Temperature 38.3°C.
11	5.11	700	0	24.5	Clinical improvement.
12	3.51	—	0	24.1	Dog normal.
13	2.66	—	0.58	24.0	
14	2.82	—	0	23.8	Normal urine output (200 cc. average).

* Feces and cage washings.

out about 200 cc. of urine per 24 hours. This gives sufficient control to show the extreme diuresis during the period of high nitrogen output following the proteose injections.

Dog 16–109.—(Table II.) Strong, old fox-terrier; male. This dog had received no proteose injection prior to the experiments, and had

been normal during a considerable period of observation in the laboratory. The details of the proteose injections are given in Table II. The proteose used in both injections was obtained from a closed loop of dog's intestine, precipitated by alcohol, and prepared in the usual way. The first injection of 25 cc. of a standard solution caused a moderate clinical intoxication with considerable increase in the total nitrogen elimination as usual. The usual temperature reaction

TABLE II.

Proteose Injections. Total Nitrogen Elimination.

Dog 16–109. Normal.

Day.	Urine.		Feces nitrogen.	Weight.	Remarks.
	Nitrogen.	Amount.			
	gm.	*cc.*	*gm.*	*lbs.*	
1	3.76	—	0	22.3	Fasting previous 4 days.
2	3.08	—	0.53	22.0	Temperature 38.9°C.
2	Proteose from dog intestinal loop. Moderate intoxication.				
3	3.76	—	0.22*	21.5	Temperature 38.7°C.
4	4.53	—	0	21.0	
5	3.58	275	0	20.8	
6	3.33	340	0	20.4	
7	3.40	250	0	20.0	
8	3.09	—	0.39	19.8	
8	Proteose from dog intestinal loop. Slight intoxication.				
9	2.56	—	0.85*	19.0	Complete recovery.
10	3.52	600	0	18.8	Temperature 38.7°C.
11	2.91	300	0	18.6	
12	2.94	—	0	18.3	

* Feces and cage washings including vomitus.

(Table VI) occurred, and there was repeated vomiting and slight diarrhea. The second injection caused only slight clinical intoxication, yet 40 cc. of the same standard solution were injected intravenously. There was a slight temperature reaction and only a trivial increase in urinary nitrogen. This is a good example of the tolerance which may develop following proteose injections. It should be emphasized that there is nothing specific about this immunity or tolerance to proteose injection.

Dog 16–46.—(Table III.) Lean, active, mongrel fox-terrier; adult male.

November, 1915. The dog was injected with a proteose obtained from the pancreas, and was slightly intoxicated. During this time and for several weeks later the animal was suffering from a moderately severe attack of distemper. A good recovery from the distemper was made in 2 or 3 weeks.

February 27, 1916. Dog in good condition. The details of the experiment are given in Table III. The first injection of proteose from a human case of general peritonitis gave no clinical symptoms of intoxication, but we cannot state whether there might not have been a rise in urinary nitrogen on the 2nd day following, as, unfortunately, a second dose of proteose from a closed intestinal loop was given. This injection caused a slight amount of clinical intoxication but a considerable rise of urinary nitrogen, which persisted for a long time.

TABLE III.

Proteose Injections. Total Nitrogen Elimination.

Dog 16–46. Immune.

Day.	Urine.		Feces nitrogen.	Weight.	Remarks.
	Nitrogen.	Amount.			
	gm.	*cc.*	*gm.*	*lbs.*	
1	2.83	—	0	25.4	Fasting previous 5 days.
2	2.95	—	0.98*	25.0	
3	2.38	—	0	24.5	Temperature 38.2°C.
3	Proteose from human peritonitis. No intoxication.				
4	2.41	—	0.67*	24.3	Temperature 38.3°C.
4	Proteose from dog intestinal loop. Slight intoxication.				
5	4.05	—	1.13	23.4	Dog active. Temperature 37.8°C.
6	4.97	—	0.84*	23.1	Clinically normal.
7	4.17	—	0	22.5	
8	3.55	—	0	22.0	
9	3.37	—	0	21.9	
10	3.40	—	0	21.8	

* Feces and cage washings.

In some experiments following a proteose injection, we have found that the curve of urinary nitrogen did not return to the original base-line during the period of observation, in spite of clinical evidence for a complete return to normal. Some of these dogs have been killed, and autopsy showed a small focus in a lung, perhaps due to an old distemper infection. Again an endocarditis has been found in several cases. Some cases may be negative, and we may be forced to assume that the body cells have been so injured that they are unable to repair the injury and return to normal during the period of observation.

TABLE IV.

Proteose Injections. Total Nitrogen Elimination.

Dog 16–114. Slight Distemper.

Day.	Urine.		Feces nitrogen.	Weight.	Remarks.
	Nitrogen.	Amount.			
	gm.	*cc.*	*gm.*	*lbs.*	
1	4.53	—	0	34.4	Fasting previous 5 days.
2	4.56	180	0	34.0	Distemper suspected.
2	Proteose from dog intestinal loop. Moderate intoxication. Vomiting, no diarrhea.				
3	5.71*	180	0	33.8	Temperature 38.6°C. Slight intoxication.
4	5.15	410	0	32.8	Temperature 38.3°C. Diuresis.
5	6.36	—	0	32.1	Dog seems normal.
6	5.14	200	0	31.8	
7	4.91	180	0	31.3	
8	4.71	200	0	31.0	
9	4.50	200	0	30.6	Note the normal after-period (nitrogen =2.85).

* Vomitus included.

Dog 16–114.—(Table IV.) Large, short-haired, mongrel; adult male. This dog was suspected of having a mild infection of distemper (*Bacillus bronchisepticus*), but the experiment was carried through and no definite clinical symptoms of distemper developed. It was not until a later metabolism period (6 months later) that it was evident that our suspicions were correct. During this last period the

base-line of nitrogen elimination was 2.85 gm. per 24 hours. We have observed several similar cases, and in certain experiments have observed a sharp rise in basal nitrogen elimination with no clinical symptoms, followed after several days of high nitrogen elimination by the usual clinical picture of distemper. This point must be always kept in mind in work of this kind on dogs that are susceptible to distemper.

It should be noted that even with the high basal excretion of urinary nitrogen, the proteose injection causes a rise in the nitrogen curve. This dog shows that the presence of one intoxication causing a high level of nitrogen excretion does not in any way interfere with the reaction following a single proteose injection.[1]

Tables V and VI give the results of three simultaneous experiments with proteose injection. The proteose was obtained in the usual way by alcoholic precipitation of dog intestinal loop fluid, and was further purified by a second alcoholic precipitation. The solution was given intravenously, 1 cc. per pound of body weight, and the clinical reactions can be followed in Table VI. The three dogs, active, strong, mongrel fox-terriers, were all in normal condition. Dogs 16–109 and 16–46 had been injected three times previously with proteose solutions, and were supposed on the basis of other experiments to have a certain degree of tolerance or immunity to subsequent proteose injections. This comes out in a study of these two tables.

The temperature reactions in practically all our experiments were followed as in these experiments (Table VI), but it is not necessary to record these observations, because the reaction following a proteose injection is so constant. These three dogs are given as characteristic examples.

Tables V and VI show several interesting points. They confirm the previous experiments, and show the great rise in total urinary nitrogen which follows an intoxication produced by a proteose. This rise in urine nitrogen may last over several days, whereas the clinical intoxication is obvious for only a day or two, and the febrile reaction is over within 12 hours.

[1] Compare Table I in the following paper, where a chronic intoxication due to an intestinal loop was combined with an injection of proteose.

It is seen that the non-immune dog shows the most intense clinical reaction to the proteose poison, and the highest rise in urinary nitrogen above the mean base-line average. The non-immune dog shows the initial delay in nitrogen elimination, the greatest rise above normal occurring in the second 24 hours. Of the two immune dogs, the larger dog (No. 16–46) shows less clinical intoxication, a higher temperature

TABLE V.

Proteose Injections. Normal and Immune Dogs. Total Nitrogen Elimination.

Day.	Dog 16–4. Non-Immune.			Dog 16–109. Immune.			Dog 16–46. Immune.			Remarks.
	Urine nitrogen.	Feces nitrogen.	Weight.	Urine nitrogen.	Feces nitrogen.	Weight.	Urine nitrogen.	Feces nitrogen.	Weight.	
	gm.	*gm.*	*lbs.*	*gm.*	*gm.*	*lbs.*	*gm.*	*gm.*	*lbs.*	
1	2.52	0	19.6	2.49	0	19.8	2.30	0	24.5	Fasting previous 4 days.
2	2.46	0	19.5	2.52	0	19.5	2.32	0	24.0	
3	2.52	0	19.4	2.46	0	19.1	2.24	0.36	23.6	
4	2.49	0	19.0	2.62*	±	18.9	2.27	0	23.3	
4	Proteose injection. Loop fluid reprecipitated by alcohol. Unit amount per pound of body weight.									
5	3.61	1.09*	18.0	2.49*	±	18.3	4.62*	±	22.5	Vomitus in cage.
6	4.42	0	17.5	3.57	0	17.9	3.22	0	22.0	
7	3.64	0	17.3	3.08	0	17.6	2.66	0	21.9	
8	2.70	0	17.0	2.44	0	17.5	2.58	0	21.6	
9	2.93	0	17.0	2.53	0	17.3	3.01	0	21.6	
Severe intoxication. Nitrogen excretion above the mean = 5.89 gm.				Moderate intoxication. Nitrogen excretion above the mean = 1.51 gm.			Moderate intoxication. Nitrogen excretion above the mean = 4.69 gm.			

* Feces and cage washings included.

reaction, and a more prompt elimination of nitrogen. It should be kept in mind that the proteose injection was proportional to body weight, and we must reduce the nitrogen figures of Dog 16–46 by 20 per cent before comparing them with the figures of Dog 16–109 or Dog 16–4. It has been shown in previous communications that dogs show

individual variations in their reaction to proteose injection, and we cannot expect the results to be uniform. It is safe to say that in general the dog which has had previous proteose injections will be less disturbed by a standard unit proteose injection, and will show less increase in urinary nitrogen above the mean base-line excretion

TABLE VI.

Proteose Injections. Normal and Immune Dogs. Temperature and Clinical Reactions.

Hour.	Dog 16–4. Non-immune.		Dog 16–109. Immune.		Dog 16–46. Immune.	
	Temperature.	Clinical reaction.	Temperature.	Clinical reaction.	Temperature.	Clinical reaction.
p. m.	°C.		°C.		°C.	
12.30*	37.9		38.4	Feces and vomitus.	38.0	Feces.
1.00	38.3	Vomitus, diarrhea.	38.5	Vomiting.	38.2	Vomiting.
1.30	37.8	More diarrhea.	38.6	Bile-stained vomitus.	39.0	Diarrhea.
2.00	37.9	Diarrhea and tenesmus.	39.1	Pulse pressure fair.	39.4	"
2.30	38.5	Vomiting continues.	39.5		39.8	Pulse pressure good.
3.00	38.9	Gelatinous diarrhea.	39.9	Diarrhea and tenesmus.	40.3	Vomiting.
3.20	39.2	Pulse tension poor.	40.1		40.4	No prostration.
4.00	39.4	Prostration, vomiting.	39.8	Vomiting.	40.6	Slight intoxication.
4.30	39.5	Severe intoxication.	39.6	No prostration.	40.2	
5.00	39.3		39.3	Recovery beginning.	39.7	
6.00	39.4	Clinical improvement.	39.1		38.9	Recovery almost complete.

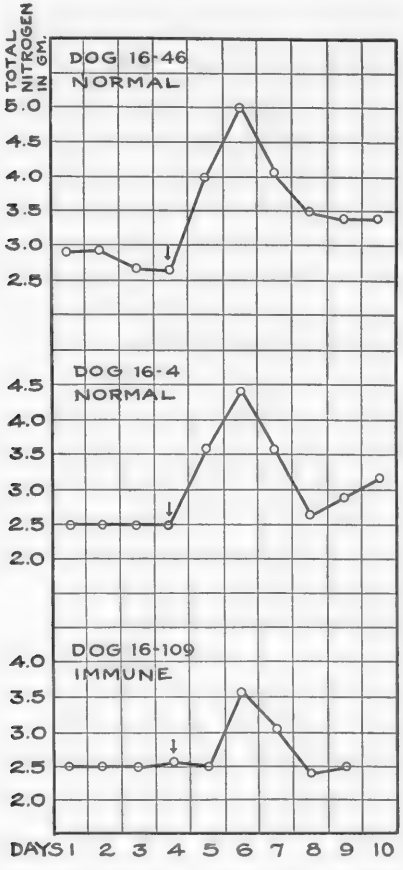
* A unit amount of pure proteose per pound of body weight was given intravenously at 12.10 to 12.20 p.m.

than the normal control (see also Tables I and II). This comes out in a more striking manner in later experiments. In Dog 16–110,[2] a lethal dose of proteose is survived with a small nitrogen increase,

[2] See Table I of the following paper.

because of the presence in this dog of a closed intestinal loop causing chronic intoxication and a high tolerance to proteose.

The temperature reaction following a proteose injection is the re-



TEXT-FIG. 1. Proteose injections. Total nitrogen elimination. The arrows indicate the injections of proteose.

sultant of two or more factors. There is a tendency for a small dose to cause a certain temperature rise and a larger dose a higher temperature, but a severe intoxication often shows a period of sub-

normal temperature during the time of prostration followed by a rise with clinical improvement (Dog 16–4). Fatal poisoning usually shows a steady fall in temperature to a low level before death. There may be a slight initial rise above normal in these fatal cases.

DISCUSSION.

The experiments tabulated above are a unit as regards several points. The fasting dog with a base-line of nitrogen excretion reacts promptly and characteristically to a toxic proteose given intravenously. The clinical reaction is violent and abrupt, but passes off rapidly. The urine shows a great increase above the base-line nitrogen elimination. The rise above normal may be present in mild intoxications on the 1st day or absent at first in severe intoxications. The second 24 hour period usually shows the most marked rise above normal, often twice normal and even three times the normal amount of nitrogen in the urine. The curve of nitrogen excretion does not fall promptly to normal, but declines slowly, and may persist for 4 to 7 days or even longer following a single injection of proteose. This fact indicates a definite tissue or protoplasmic injury which is not adjusted promptly.

There is usually more or less diuresis at this time, and in some experiments a remarkable rise in urine volume (Table I) is noted. The urine volume was not carefully studied in these particular experiments, but this diuresis comes out more clearly in experiments to be published in the near future.

To permit of a clean-cut discussion of this subject of proteose intoxication, it is necessary to recall a few facts which are easy to demonstrate by the injection of a toxic proteose in a normal dog. A small injection of proteose may be given through a hypodermic needle in the jugular vein without anesthesia. The initial reaction is almost instantaneous and within a second or two, even before the injection is completed, the dog will show salivation. Within 5 or 10 minutes the dog may begin to vomit bile-stained mucus. This will continue, depending upon the amount of proteose injected, and feces will soon be passed. Diarrhea may become evident within an hour or less.

The blood pressure reaction may not appear for over an hour, whereupon a progressive fall in blood pressure and temperature may be noted in severe cases. Mucus and blood-tinged diarrhea may appear at this time, especially in cases which end fatally within 6 hours. In some instances the blood pressure reaction may be as prompt as the vomiting. It is possible that the ether anesthesia used in some experiments may explain some of this delay in the toxic reaction.

The blood often becomes incoagulable when tested *in vitro* following a large proteose injection. An excess of antithrombin can be demonstrated in these cases. This is not an absolutely constant finding, and is usually not demonstrable after small doses of proteose, particularly if the injection is made slowly. Too much emphasis should not be placed upon this factor.

A characteristic feature of autopsies of fatal cases of acute proteose intoxication is the splanchnic engorgement (3). This is well shown in the spleen, liver, and pancreas. The mucosa of the small intestine, particularly the duodenum, shows this reaction best—a velvety purple engorged mucosa.

Blood concentration is the rule in almost all cases of acute proteose intoxication. The blood is almost syrupy, and yields only one-fourth or less of its total volume as plasma when drawn into oxalate. Normal dog blood drawn into oxalate (1 part to 9) yields slightly more than one-half by volume of plasma, using the usual speed centrifuge. One's first thought is to explain this blood concentration by the vomiting, diarrhea, and consequent loss of fluid. But it is possible to produce such an acute proteose intoxication that there will be scarcely any loss of fluid by vomiting or diarrhea. A large dose (two or three times a minimum lethal dose) of proteose may be given intravenously and cause death within 2 hours with practically no vomiting and not a trace of feces. These cases may show dry, serous surfaces and a blood which is syrupy and will yield per 100 cc. scarcely 5 to 10 cc. of plasma on centrifuging the oxalated blood. This is almost conclusive proof that much of the circulating fluid has been taken up by the body tissues. This evidence for a definite tissue injury should not be lost sight of in the discussion which follows.

We may now ask: How does the proteose act upon the individual?

Does this toxic proteose act on the blood plasma, on the blood cells, or upon the body tissues, or both plasma and cell protein? That the proteose can cause marked changes in metabolism and all grades of intoxication is obvious.

The work of Jobling, Petersen, and Eggstein (4, 5) and others is usually interpreted to mean that the most important changes take place in the blood plasma. We believe that their work can be interpreted in the light of a profound cell injury and liberation of the ferments into the blood. Experiments with blood serum or plasma *in vitro* do not disprove this point. Their work shows a mobilization of ferments demonstrable in the blood following proteose injections and other intoxications. There is a fluctuation of the ferment-antiferment balance with periods of protease activity, which is believed to act upon serum proteins with the formation of toxic substances or split products.

We wish to point out again the evidence that there is a definite tissue reaction following a large toxic dose of proteose. The blood plasma is greatly concentrated without any marked loss of fluid from the body, and it may be assumed that the body protoplasm has taken up much fluid from the blood. It may be argued that the blood plasma reaction is primary, and the tissue reaction is secondary, or *vice versa*. More evidence is needed to settle this point, and it is possible, if not probable, that both body cells and body fluids react to the initial proteose injection.

The long continued excretion of excess nitrogen after a single proteose injection, a period of 3 to 7 days or even longer, speaks for an essential injury to cell protoplasm somewhere in the body. We have no histological evidence of this injury, but it is now well recognized that a cell may be profoundly disturbed as to function without showing any structural variation from normal which we can recognize with present methods. We have no evidence that any particular organ or tissue is picked out by this toxic proteose, but body cells which are especially rich in ferments may be the most susceptible.

For the sake of visualizing the reaction of this proteose upon cell protoplasm, we may assume that the protoplasm is in delicate equilibrium, perhaps a ferment-antiferment equilibrium or colloidal dis-

persion equilibrium. The toxic proteose may shock or disturb this delicate physical balance, and a multitude of reactions may take place. The protoplasm may take up available water—hydration of a colloid. This phenomenon may be the cause or effect of the protoplasmic injury, but it is fairly definite. There is no evidence that this reaction is reversible, but the chain of events when once started follows a well recognized sequence. It has been shown (6) that a short time after a proteose injection the blood of the poisoned dog is inert toward a normal dog. This again emphasizes the body cells as the elements attacked by the proteose, which is so rapidly withdrawn from the blood stream.

Whatever the word picture we use—ferment-antiferment balance, physical or colloid balance, or any other—we mean that there is evidence of a direct injury of cell protoplasm by this toxic proteose. No efficient method of restoring this balance or undoing the cell injury has been discovered. We know that the body is able to bring conditions back to normal if the shock has not been too great, but we do not understand this mechanism. We have previously submitted evidence (7) again in favor of the activity of the tissue and organ cells. Repeated injections of sublethal doses of proteose bring about a certain degree of tolerance or immunity in dogs (7). The body fluids of these dogs are inactive toward toxic proteoses, but the organ emulsions and ferment-containing organ extracts of these dogs destroy *in vitro* the toxic proteoses. It is extremely important to study every phase of this reaction in the immune animal, for we may by such means gain an understanding of the mechanism of body defense. This knowledge may be of great value in the treatment of acute intoxications.

It has been noted that following an injection of proteose, there may be a latent period of an hour or more before the grave toxic reaction with prostration sets in. The blood removed at this time from an intoxicated dog is inert toward a normal animal. These two facts suggest an initial injury to cell protoplasm, which starts some intracellular reaction resulting in protein splitting with the formation of more toxic substances and the final overwhelming of the host. This may be spoken of as a vicious circle, where the equilibrium between the arc of the vicious circle and the straight line of normal

balance is extremely delicate. If the body reserve cannot restore this balance, the cells may actually form substances from their own protoplasm which are fatally toxic. The physical condition of the cell may be so changed from normal without any histological change that cell life and metabolism are no longer possible.

It is well known that a proteose injection will cause great destruction of white cells, but no appreciable red cell injury. It may be assumed that this is primary or secondary to the other reactions which have been mentioned. It is also possible to look upon this as further evidence of cell injury by this toxic proteose—an upset in the delicate protein equilibrium in these cells with disintegration and perhaps further production of toxic split products. It is known that white cells are rich in ferments,—so too are liver cells and the epithelial cells of the pancreas and intestinal mucosa.

The tabulated experiments of this paper add some facts concerning the tolerance or immunity which develops in dogs after repeated sublethal doses of proteose. It is clear that the second or third unit dose of proteose will cause, as a rule, much less violent clinical reactions (Tables II and VI). In general, we may say that the immune dog will put out less urinary nitrogen than a similar control dog after a unit injection of toxic proteose. The clinical features and excess of nitrogen elimination are usually in accord;[2] the reactions tend to run parallel, but we are inclined to believe that the curve of nitrogen excretion gives a more accurate index of body injury caused by the proteose than do the clinical reactions.

SUMMARY.

Proteose injections in dogs cause vomiting, diarrhea, temperature reactions, low blood pressure, prostration, and, after large doses, an excess of antithrombin with incoagulable blood.

A single proteose injection, for example one-half a lethal dose, causes abrupt clinical reactions in a normal dog with apparent complete recovery within 24 to 48 hours.

The nitrogen elimination curve in a fasting dog under such conditions shows a great rise in total urinary nitrogen. The apex of the curve usually falls during the second 24 hour period following

the injection. This rise may be over 100 per cent increase above the mean base-line nitrogen level. It does not fall promptly to normal but declines slowly in 3 to 5 days or more toward the original base-line (Text-fig. 1). This speaks for a definite cell injury with destruction of considerable protein substance due to a single proteose injection. The disturbance of cell equilibrium is not rapidly or promptly restored to normal.

A dog which has received previous proteose injections is partially immune or tolerant to subsequent injections of proteose. These dogs, as a rule, show less intense clinical reactions and less rise in the curve of nitrogen elimination following a unit dose of standard proteose as compared with normal or non-immune controls.

The proteose used in these experiments was prepared as described from material obtained in cases of intestinal obstruction or of closed intestinal loops.

These experiments explain the sharp rise in blood non-protein nitrogen which follows within a few hours the injection of a toxic proteose. They also point to the correct explanation of the high non-protein nitrogen of the blood found in intestinal obstruction or with closed intestinal loops.

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PROTEOSE INTOXICATIONS AND INJURY OF BODY PROTEIN.

II. The Metabolism of Dogs with Duodenal Obstruction and Isolated Loops of Intestine.

By G. H. WHIPPLE, M.D., J. V. COOKE, M.D., and T. STEARNS, M.D.

(From the George Williams Hooper Foundation for Medical Research and the Departments of Pathology and Surgery of the University of California Medical School, San Francisco.)

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The results which follow the injection of toxic proteoses in normal and immune dogs have been reported in a previous communication.[1] The proteose material was obtained from intestinal obstruction or from closed loops of the intestine. The present paper deals with the reaction which develops in the dog following intestinal obstruction of a certain type or the isolation of closed intestinal loops. It is clear that the curves of nitrogen elimination show the same high rise above normal following intestinal obstruction as recorded above, as after an injection of a toxic proteose. We have long believed that the intoxication associated with isolated intestinal loops and intestinal obstruction is due to the absorption from the intestinal mucosa of a toxic proteose similar to the proteose which can be isolated from the closed loops or obstructed intestine. These experiments give further support to this belief, and add other facts which are of value for a proper understanding of the various proteose intoxications.

Methods.

Dogs were used in all the experiments. All operations were done under complete surgical ether anesthesia with the usual aseptic technique. The various procedures for the collection of urine, analysis, etc., are described in detail in the preceding paper.

[1] Whipple, G. H., and Cooke, J. V., *J. Exp. Med.*, 1917, xxv, 461.

EXPERIMENTAL OBSERVATIONS.

Dog 16–110.—(Table I.) Strong, black cocker spaniel; adult male.[2]

Feb. 15, and Feb. 21, 1916. Proteose injections were given as described. Both caused a definite intoxication, but the second dose was larger without causing any more marked intoxication, an evidence of established tolerance.

Mar. 29. The dog is completely recovered from previous experiments, and should have a moderate tolerance or immunity against proteose. It has been established that these dogs survive a closed loop for a longer time than a non-immune animal (1). The metabolism experiment was started and carried on as usual (Table I).

Mar. 31. Ether anesthesia and operation to produce the usual closed loop of jejunum. The larger part of the jejunum was isolated, the ends turned in, and the small intestine united around it by an end to end anastomosis. Time of operation, 1½ hours. The dog made a good recovery, and gave no signs of acute intoxication as did Dog 16–156 (Table II). There was no complicating vomiting and little feces.

Apr. 16. Dog shows no clinical signs of intoxication. Visible peristalsis seen for the first time. This indicates some filling of the closed loop.

Apr. 20. Several feedings (Table I) of sugar and vegetable fat were given to study the amount of protein-sparing effected by this means. These experiments and others indicate that under this condition of chronic intoxication from a closed loop, the protein-sparing action of carbohydrates is less marked than in a normal dog.

May 1. Condition unchanged. Visible peristalsis is not conspicuous, but it is easy to see. Intravenous injection of a standard proteose obtained from a closed loop of intestine.

A control dog was given the same dose per pound of body weight with a rapidly fatal result. The autopsy findings were typical of acute proteose intoxication.

This dog (No. 16–110) had the most marked tolerance to proteose which we have noted in all our experiments. He also survived a closed loop of the jejunum longer than any animal in a large series, so we may assume that he was highly tolerant because of the continued slow intoxication from the closed intestinal loop. He was protected against the acute initial intoxication of the loop by the previous injections of proteose.

This lethal dose of proteose was given to Dog 16–110 without any definite signs of intoxication. Following the injection there was

[2] The first proteose injections in this dog are recorded in Table I of the preceding paper.

a slight febrile reaction and one attack of vomiting. The dog passed soft feces, but there was no diarrhea. The pulse pressure was excellent. The elimination of nitrogen in the urine indicates the same lack of definite reaction, and we note only a trivial rise in total nitrogen. Compare with this the reaction of this dog when injected with a fraction of a lethal dose of proteose obtained from a case of human intestinal obstruction.[2]

May 6. Ether anesthesia, operation, and removal of the closed loop of jejunum. The dog recovered and is in normal condition at present. The closed loop contained thick, pasty material in considerable amount. This material was diluted, and preserved for further study. Total nitrogen of loop fluid, 5.21 gm. This should be compared with the fluid which accumulated rapidly in a similar loop in Dog 16–156 (Table II). This fluid was more abundant as the loop was longer and more acutely distended (Dog 16–156), yet it contained only 2.21 gm. of nitrogen. The relative toxicity of the two materials has not as yet been tested.

The loop shows an intact mucosa, which in general is pale, but shows a few indefinite areas of slight congestion. Microscopic sections show a normal mucosa which is covered with a dense gelatinous layer of material containing a few degenerated cells and nuclear particles, but for the most part is homogeneous, like gelatin. This is the thick, adherent loop material which is so rich in the toxic proteose. The villi and epithelial cells covering the villi are normal.

The preceding experiment (Dog 16–110, Table I) merits careful consideration for several reasons. Reference to the first proteose injections in this dog[2] shows the usual reaction to proteose and some evidence of immunity or tolerance to the second injection.

1 month later, after complete recovery, this dog was operated upon, and a closed loop of jejunum produced. It is well known that these dogs suffer a characteristic intoxication, and it has been shown also (1) that dogs previously injected with proteose are less susceptible to the acute intoxication of a closed intestinal loop. Dog 16–110 with a closed intestinal loop showed a chronic type of intoxication rather than the acute reaction usually seen in non-immune dogs, presumably due in part to the proteose injection 1 month before—compare the acute intoxication of the next experiment (Table II) in a non-immune dog.

The clinical signs of proteose intoxication in Dog 16–110 are slight or absent, and the curve of nitrogen elimination is of considerable in-

TABLE I.

Dog 16–110. Long Closed Loop of Jejunum. Total Nitrogen Elimination. Immune to Proteose.

Day.	Urine.		Feces nitrogen.	Weight.	Remarks.
	Total nitrogen.	Amount.			
	gm.	*cc.*	*gm.*	*lbs.*	
1	2.20	200	0	26.63	Fasting previous 3 days.
2	1.99	—	0.31*	26.13	
2	Long loop of jejunum isolated.				
3	2.35	150	0	26.13	Temperature 38.8°C.
4	4.23	—	0.17	—	Vomitus in urine.
5	4.27	600	0	25.06	No vomitus.
6	3.56	600	0	24.44	Temperature 38.6°C.
7	2.86	455	0	24.19	" 38.5° " Wound swollen.
8	3.02	460	0	23.88	Clinically normal.
9	2.72	400	0	23.44	Wound firm.
10	2.38	515	0	23.31	
11	2.30	540	0	22.75	
12	2.44	410	0	22.56	
13	2.41	440	0	22.44	
14	2.20	565	0	22.19	
15	2.07	370	0	21.94	
16	2.13	460	0.39	21.81	
17	2.16	425	0	21.31	
18	2.24	450	0	21.25	Intestinal peristalsis visible.
19	1.99	450	0	20.88	
20	2.02	400	0	20.69	
21	1.90	405	0	20.56	
22	2.02	335	0	20.44	4 p.m. Fed 50 gm. of milk sugar, 100 gm. of vegetable fat ("Crisco").
23	1.90	345	0	20.50	Fed milk sugar and fat.
24	1.68	295	0	20.50	
25	1.90	455	0	20.19	
26	2.24	355	0.14	20.0	4 p.m. Fed 15 gm. of milk sugar, 40 gm. of vegetable fat ("Crisco").
27	2.10	390	0	19.81	
28	1.83	355	0.10	19.69	12 m. 100 gm. of milk sugar by stomach tube.
29	2.97*	—	±	19.44	Diarrhea.
30	1.88	465	0	19.06	4 p.m. 50 gm. of cane sugar by stomach tube.

* Feces and cage washings.

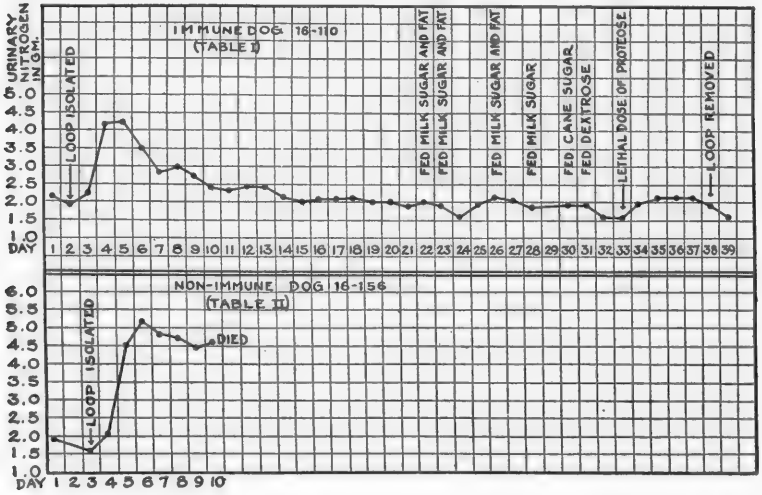
TABLE I—*Concluded.*

Day.	Urine.		Feces nitrogen.	Weight.	Remarks.
	Total nitrogen.	Amount.			
	gm.	*cc.*	*gm.*	*lbs.*	
31	1.83	750	0	19.0	1 p.m. 50 gm. of dextrose by stomach tube.
32	1.65	740	0	18.94	
33	1.68	272	0	18.56	Visible peristalsis in abdomen.
33	Proteose from intestinal loop. Control dose fatal for normal dog.				
34	1.93	450	0.55	18.06	Temperature 38.1°C.
35	2.24	320	0	17.69	" 38.3° "
36	2.24	320	0	—	
37	2.21	335	0	17.44	
38	1.88	265	0	17.13	Temperature 38.2°C.
38	Closed loop of intestine resected. Loop contents total nitrogen 5.21 gm.				
39	1.64	160	—	16.19	Recovery.

terest. There is a sharp initial rise following the operation, but then a gradual fall toward the normal base-line elimination which is reached at the end of the 3rd week. A part of the initial rise is due to the wound reaction, and this is accurately controlled in Table VI, where the same dog after a considerable interval with recovery to normal was used for a control laparotomy experiment. The greater part of the rise in urinary nitrogen above the base-line (Text-fig. 1) is due to the intoxication associated with the closed loop of jejunum.

During this entire experiment, the dog was fasting. He returned to a normal or almost normal base-line nitrogen elimination during the 3rd week. There are no clinical signs of intoxication, but we note the presence of peristalsis in the abdomen—a sign that the loop is filling up. Sugar and fat feeding suggest an abnormal condition in this dog (Table I); they do not depress the curve of nitrogen elimination as in normal controls. There is not the same protein-sparing action. These experiments are merely suggestive, and more detailed work now in progress is required to clear up this point. It is possible that these experiments may throw light on the subject of protein-carbohyrate metabolism in disease.

Finally a lethal dose of proteose was given to this dog with little, if any, clinical reactions and a trifling rise in the curve of nitrogen elimination. This indicates a maximum tolerance for proteose which could scarcely arise except following a long continued chronic proteose intoxication. We assume that the presence of the closed intestinal loop was responsible for this chronic intoxication, which gave this dog such a high degree of tolerance to proteose injection. An occasional injection of proteose will give a certain degree of tol-



TEXT-FIG. 1. Closed loop of jejunum. Total nitrogen elimination of immune and non-immune dogs.

erance, as is seen in the first experiments on this dog, but this tolerance or immunity is relatively slight and transient (1), and cannot explain such a degree of protection as is noted after this injection of a lethal dose of proteose.

Table II (Dog 16–156) gives a good example of the nitrogen elimination and intoxication which may follow an experimental closed intestinal loop in a normal, non-resistant dog. It should be noted that the nitrogen elimination rises well over 100 per cent above normal,—almost 200 per cent above the normal base-line on 1 day. The

complicating wound infection was present only during the last 2 days, and may have helped a little in the acute intoxication which was more rapidly fatal than usual.

TABLE II.

Dog 16–156 (Non-Immune). Long Closed Loop of Jejunum. Total Nitrogen Elimination.

Day.	Urine.		Feces nitrogen.	Weight.	Remarks.
	Total nitrogen.	Amount.			
	gm.	*cc.*	*gm.*	*lbs.*	
1	1.85	40	0	13.75	Fasting previous 4 days.
2	1.29*	—	±	13.56	Some urine lost.
3	1.68	70	0	13.31	
3	Long loop of jejunum isolated.				
4	2.27	515	0.43*	12.44	Vomitus in urine. Temperature 39.2° C.
5	4.48	650	0	12.06	" " "
6	5.21	1,260	0	12.00	Some urine lost. Temperature 38.6° C.
7	4.84	235	0	11.81	Vomitus in urine.
8	4.76	150	0	11.50	" " "
9	4.45	125	0.89*	11.06	No vomitus. Infected wound.
10	4.64	130	0	10.75	" " Temperature 37.9° C.
11	1.13	80	0	—	Died during previous night.

* Feces and cage washings. Loop contents total nitrogen 2.21 gm.

Dog 16–156.—(Table II.) Fox-terrier; adult male.

May 9, 1916. Metabolism experiment started (Table II).

May 12. Ether anesthesia and operation to produce the usual closed loop of intestine. The better part of the jejunum was isolated, the ends turned in, and the small intestine united around it by means of an end to end anastomosis. Time of operation, 1½ hours.

May 13–14. The dog shows a good deal of acute intoxication, and vomits repeatedly. Wound looks well.

May 15–16. Intoxication still marked.

May 18. Wound is somewhat infected, and the skin edges are broken down.

May 19. Dog is sick and temperature falling. Died in night. Autopsy next morning. The abdominal wound shows superficial infection, but the deeper layers are clean. The peritoneum is clean, but there is a slight excess of fluid.

Thorax, heart, and lungs are all normal. Liver and kidneys show cloudy swelling. Spleen is small. Urinary bladder is empty, and shows no inflammation. The entero-enterostomy is patent and well healed. The gastro-intestinal tract is normal.

The loop of jejunum measures 107 cm. in length. It is acutely distended and deep red. It contains 155 cc. of thick, slimy, reddish slate-colored fluid. The mucosa is reddish and velvety throughout. The ends of the loop are well closed. The loop fluid contained 2.21 gm. total nitrogen. The loop of intestine produced in this experiment (Table II) is similar to that of the preceding experiment (Table I). This dog (No. 16–156) was normal, and had received no proteose injections. Perhaps partly because of this he developed an acute intoxication, which rapidly led to a fatal termination. Table II contrasts the relatively acute intoxication with the more chronic poisoning noted in Table I in the dog which had been previously injected with proteose.

TABLE III.

Dog 16–129. Duodenal Obstruction. Total Nitrogen Elimination.

Day.	Urine.		Feces nitrogen.	Temperature.	Weight.	Remarks.
	Nitrogen.	Amount.				
	gm.	*cc.*	*gm.*	*°C.*	*lbs.*	
1	1.75	80	0	—	16.06	Fasting previous 4 days.
2	1.65	150	0	—	15.75	
3	1.79	80	0	—	15.62	
4	1.62	80	0.50	—	15.31	
4	Duodenal obstruction. Gastrojejunostomy.					
5	2.38	—*	0	38.4	14.44	Good recovery.
6	3.95	800*	0	38.5	14.06	Definite intoxication.
7	4.87	—*	0	37.9	13.75	
8	4.76	400*	0	37.3	13.44	Sick.
9	4.84	405*	0	—	13.0	
10	4.90	270*	0	37.5	12.56	Little vomitus.
11	3.44	165*	0	36.4	12.06	Subcutaneous abscess.
11	Killed. Autopsy performed at once. Blood non-protein nitrogen, 158 mg. per 100 cc.					

* Vomitus and cage washings.

Dog 16–129.—(Table III.) Active, young fox-terrier; male.

Mar. 11, 1916. The dog had recovered from a mild attack of distemper. He was given a proteose injection of standard material prepared as usual from a closed loop of intestine. There were a moderate temperature reaction and some evidences of intoxication.

Apr. 8. The dog is in good condition (Table III).

Apr. 11. Ether anesthesia and operation as usual to produce duodenal obstruction. Time of operation, 1½ hours. The jejunum about 1 inch beyond

the duodenojejunal junction was cut across, and the upper end closed by inversion. The lower cut end was joined to the stomach, and a gastrojejunostomy performed as usual.

This dog (No. 16–129) shows an acute intoxication following this type of duodenal obstruction with gastrojejunostomy. The picture is somewhat complicated by an infected abdominal wound with an abscess developing just before death.

Apr. 18, 1 p.m. Dog is very sick. Ether anesthesia and bleeding from carotid.

The blood shows 158 mg. of non-protein nitrogen per 100 cc. and 70 mg. of urea nitrogen—or 44 per cent of total nitrogen.

There is an abscess in the subcutaneous tissue near the wound. It extends toward the costal margin, and contains 3 to 5 cc. of pus. There is little edema, and the edges of the wound are closed and well healed. The peritoneum is clean and moist. The thorax, heart, and lungs are normal. The liver, pancreas, spleen, kidneys, and bladder show nothing of importance. The stomach is slightly dilated, and contains bile-stained fluid. The gastrojejunostomy is patent, and the small intestine shows slight congestion only. The stomach shows several superficial erosions not over 0.5 cm. in diameter, one in the antrum and two close to the gastrojejunostomy. The duodenum is slightly dilated with bile-stained fluid. Its mucosa is intact but slightly congested. The lower end is well closed.

Dog 16–136.—(Table IV.) Black cocker spaniel; adult female.

Mar. 21, 1916. Splenectomy done as usual; rapid recovery.

Apr. 17. Experiment begun; the details are recorded in Table IV. Dog in normal condition. Fasting for previous 3 days.

Apr. 20. Ether anesthesia and operation as usual to produce a duodenal obstruction at the junction of the duodenum and jejunum with a gastrojejunostomy. The operation was identical with that in the preceding experiment (Dog 16–129); duration 1½ hours.

The clinical course of this animal is less acute than the preceding, and there are several days which show no vomitus, and give confirmatory data to show that the loss of nitrogenous material in the vomitus is slight, and can be neglected without introducing an appreciable error.

May 4, 2 p.m. Died. Autopsy performed at once. The serous cavities are negative. The parenchymatous organs show nothing of importance. The stomach and duodenum are somewhat distended, and show an intact mucosa. The gastrojejunostomy shows a large opening, and is efficient. The dog died from simple intoxication with no complicating factors except the splenectomy which will not be discussed at this time.

The two preceding experiments (Tables III and IV) deal with a type of duodenal obstruction which is of considerable value in experimental work. A simple obstruction at the duodenojejunal

TABLE IV.

Dog 16–136. Duodenal Obstruction. Total Nitrogen Elimination.

Day.	Urine.		Temperature.	Weight.	Remarks.
	Nitrogen.	Amount.			
	gm.	*cc.*	*°C.*	*lbs.*	
1	1.98	100	—	22.75	Splenectomy previously.
2	1.65	110	—	22.56	
3	1.93	154	—	22.31	
3	Duodenal obstruction. Gastrojejunostomy.				
4	2.74	300	38.3	21.75	Good recovery.
5	2.98	800*	—	20.68	Vomitus.
6	5.17	500*	—	20.75	Little vomitus.
7	4.62	180*	38.0	20.31	" "
8	3.63	250*	—	20.06	" "
9	3.64	150	—	19.81	No vomitus.
10	4.14	330*	37.9	19.38	Little vomitus.
11	3.19	300	38.3	19.19	No vomitus.
12	4.42	231	38.1	18.62	" "
13	2.74	285	—	18.25	" "
14	2.49	245*	38.1	17.94	Little vomitus.
15	3.30	240	37.3	17.56	A little feces.
16	3.33	250	36.8	17.25	No vomitus.
17	1.42	175	35.8	17.0	" "
17	Died; autopsy.				

* Vomitus and cage washings.

junction in a dog will lead to a fatal outcome usually in from 3 to 6 days with much vomiting and loss of fluid. The type of duodenal obstruction used here (Tables III and IV) gives a more chronic form of intoxication with little vomiting and loss of fluid—a condition much more desirable for experimental work. A complete obstruction of the duodenum is effected at its lower end by section and inversion of the intestine. The jejunum is joined to the stomach by a gastrojejunostomy, which allows fluid from the stomach to escape into the small intestine, and has much to do with the prevention of vomiting and the consequent loss of body fluids. There is present with this type of duodenal obstruction a definite intoxica-

tion, as we see from an examination of Tables III and IV. Under these conditions a dog may live from 1 to 3 weeks, and show little tendency to vomit, yet there are definite signs of intoxication, and such dogs can be shown to have a definite tolerance to proteose injections, as was shown in the case of closed loop experiments (Table I).

Both experiments show a great rise above the base-line of nitrogen elimination in the urine. The first experiment (Dog 16–129, Table III) shows a more acute course and a higher nitrogen curve. The second experiment (Dog 16–136, Table IV) shows a more chronic intoxication and a nitrogen curve which is quite high and sustained considerably above normal up to the end. The second experiment shows little loss of fluid by vomitus, and gives a pretty good control of this factor.

Tables V and VI give the necessary controls for the operative procedures employed in the preceding experiments. Similar experiments to control ether anesthesia and simple laparotomy have been done with identical results, and need not be reported.

TABLE V.

Dog 17–10 (Normal). Laparotomy Control. Total Nitrogen Elimination.

Day.	Urine.		Feces nitrogen.	Temperature.	Weight.	Remarks.
	Total nitrogen.	Amount.				
	gm.	*cc.*	*gm.*	°C.	*lbs.*	
1	1.99	—	0	38.3	17.0	Fasting previous 4 days.
2	2.07	64	0	38.3	16.63	
3	1.95	60	0	38.3	16.44	
3	Laparotomy control (midline). Ether for 30 minutes.					
4	2.81	270	0	38.9	—	Little vomitus.
5	3.14	165	0	38.6	15.94	Wound edematous.
6	2.85	135	0	38.7	15.63	
7	2.52	90	0	38.6	15.38	Wound healing well.
8	2.32	70	0	38.7	15.31	
9	2.36	70	0	38.2	15.0	
10	2.50	100	0	38.1	14.75	Wound firm.
11	2.09	95	0	38.1	14.69	
12	1.96	92	0	38.3	14.38	Normal.

TABLE VI.

Dog 16–110. Ether Anesthesia and Laparotomy Control. Total Nitrogen Elimination.

Day.	Urine.		Feces nitrogen.	Temperature.	Weight.	Remarks.
	Total nitrogen.	Amount.				
	gm.	*cc.*	*gm.*	°C.	*lbs.*	
1	2.11	210	0	38.9	19.5	Fasting previous 10 days.
2	1.97	230	0	—	19.13	
3	1.75	188	0	38.2	19.06	
3	Surgical anesthesia. Ether for 1 hour.					
4	2.27	250	0	38.0	18.75	Good recovery.
5	1.69	195	0	37.9	18.25	
6	2.00	215	0	38.0	18.06	
7	1.89	220	0	37.9	17.94	
7	Laparotomy control. Rectus incision. Ether for 30 minutes.					
8	2.58	200	0	37.7	17.56	Good recovery.
9	2.55	365	0	—	17.50	Diuresis.
10	2.39	340	0	37.8	17.06	Wound slightly swollen.
11	2.23	275	0	37.8	16.88	
12	1.93	235	0.22	37.3	16.69	Wound firm.
13	2.00	270	0	37.7	16.44	Normal.

Ether anesthesia for 1 hour causes only a trivial rise in the curve of nitrogen elimination in the urine. In some cases the increase may be so slight as to be doubtful, and fall within the fluctuation due to the methods of collection and analysis.

Laparotomy was done in the usual manner, either in the midline or through the rectus. A few coils of intestine were brought out and handled as in preparation for an intestinal anastomosis. The incision was closed by silk and catgut, as in the other operations. The total increase in urinary nitrogen above the base-line after a laparotomy corresponds closely to the same rise noted after the injection of a single dose of toxic proteose.[3] We may assume with considerable certainty that the injured tissue which results from the

[3] See Table II of the preceding paper.

incision and closure by ligatures plus the slight bacterial infection of the clean wound can cause a suitable disintegration of tissue protein to form certain toxic split products which are responsible for this general reaction.

Histologically we may recall that a wound healing *per primam* is in reality a thin linear abscess with all the ingredients of the true abscess—tissue necrosis, skin bacteria, and wandering cells. There is no massing of cells sufficient to produce gross evidence of tissue and cell solution (pus), but we know that the injured cells go to pieces in the wound, and are removed by solution or phagocytosis. We may assume that this process is capable of forming various protein split products—probably among others a little toxic proteose which may be in part responsible for the general reaction and the increase in nitrogen elimination.

We may anticipate a report to appear in the near future dealing with various types of abscess, and say that this reaction produced by a clean wound is multiplied several times by an abscess reaction; but in all essentials the reactions are alike, the difference is merely quantitative. Further, it is possible to isolate toxic proteose-like substances from the pus of these abscesses.

DISCUSSION.

The experiments described above throw some light on the intoxication which appears in association with isolated intestinal loops or intestinal obstruction. There is a great increase in the urinary nitrogen above the fasting base-line level, and this rise in nitrogen elimination is in general proportional to the severity or acuteness of the intoxication. This great increase in urinary nitrogen indicates a considerable breaking down of body protein, and this destruction of protein is probably an important part of the intoxication. This explains in great measure the rise of non-protein nitrogen in the blood which has been observed in these conditions (2).

Tolerance or immunity to proteose intoxication develops in these experimental conditions (closed loops or intestinal obstruction) and with this tolerance we note a tendency for the nitrogen curve to fall toward normal (Table I). Dogs that have been injected repeatedly with sublethal doses of toxic proteose show less evidence of protein

injury and a lower curve of nitrogen elimination[4] than normal controls. These dogs are tolerant to proteose injections, and also are much more resistant to the intoxication following intestinal obstruction or closed intestinal loops.

In Tables V and VI there is evident a definite diuresis which deserves some consideration. This diuresis is observed too, after single injections of proteose, but is effectually masked by the vomitus in the obstruction experiments. These dogs had access to water at all times, and in the main the observations indicate simply an increased thirst. Perhaps we may say the tissues craved water. In order to determine whether there is actual retention of fluid during periods of acute intoxication and elimination of this excess during later periods, we must perform more carefully controlled intake experiments with sufficiently chronic intoxication so that there may be no tendency to vomit. We have submitted some evidence in the preceding paper to show that in acute proteose intoxication the body protein takes up water from the blood. If recovery takes place, we must assume that this excess water will be thrown out by the body cells. The thirst noted in these intoxications may be one sign that the proteins of the body need more water. This delicate balance between the cell protein or colloids and the water of the blood will require much more study before we have an intelligent understanding of a few of these important phenomena. The proteose intoxications, too, may offer a valuable means for further study of these phenomena.

Table I gives some data on the protein-sparing action of carbohydrates and fat in a chronic intoxication associated with an isolated intestinal loop. These few preliminary observations merely suggest that the protein-sparing action of carbohydrates and fat is not so definite in chronic proteose intoxications as in normal fasting dogs. We hope to report further on this point, and there are obviously many possibilities which will call for further experimental work. These conditions of proteose intoxication do not show any evidence of acidosis.[5]

[4] See Text-fig. 1 of the preceding paper.

[5] Certain of these experimental animals were included in a series of observations made by Dr. Whitney, and will be reported by him in detail in a separate publication.

SUMMARY.

Dogs with isolated loops of small intestine show many evidences of intoxication. A study of the total nitrogen elimination shows a great rise above the normal base-line minimum of the fasting period (Table II). This means that the intoxication is associated with a great destruction of body protein, and explains the high non-protein nitrogen of the blood which was observed and reported previously (2).

Injection of a proteose obtained from a closed intestinal loop will cause a similar rise in the nitrogen elimination curve. This furnishes more evidence that the intoxication observed in association with a closed intestinal loop is in reality a proteose intoxication.

Dogs injected with sublethal doses of proteose will show a definite tolerance to subsequent injection, and will show much less acute intoxication after the isolation of a closed intestinal loop (Table I). These immune or tolerant dogs show a much less pronounced rise in the nitrogen elimination curve during proteose intoxication of any type. This indicates that the tolerance or immunity to proteose gives more protection for the body proteins against the injury which these toxic proteoses inflict upon the body cells.

Complete duodenal obstruction combined with a gastrojejunostomy gives a chronic type of intestinal obstruction associated with little vomiting, which is peculiarly suited to metabolism study (Table IV). Such duodenal obstructions show a definite and sustained rise in the curve of nitrogen elimination above the normal base-line level. These dogs, too, are tolerant to injections of standard toxic proteoses.

Control ether anesthesia experiments show little if any rise in the curve of nitrogen elimination (Table VI).

Control laparotomy experiments show a definite rise in the curve of nitrogen elimination, but a rise which is small compared with the rise noted in the intoxication of duodenal obstruction or of isolated intestinal loops. It is probable that the tissue injury and disintegration associated with the wound reaction are responsible for the general reaction. We may assume that protein split products from the wound area are absorbed and are responsible for the general reaction observed.

We propose to assume that the intoxications here studied are

associated with a definite proteose intoxication, which is capable of initiating and continuing a profound injury of tissue protein. One index of this protein injury is the great and sustained rise in the curve of total nitrogen elimination.

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THE EFFECT OF COLD UPON MALARIA PARASITES IN THE MOSQUITO HOST.

By W. V. KING, Ph.D.

(*From the Bureau of Entomology, U. S. Department of Agriculture, Washington, D. C., and the Laboratories of Clinical Medicine of the School of Medicine of Tulane University, New Orleans.*)

Plates 38 and 39.

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The impression is gained from the literature on malaria that the development of malaria parasites in *Anopheles* is arrested at a temperature of about 60° F. and that the parasites themselves are destroyed at temperatures below this. The question is of importance since it involves the survival of infection in mosquitoes after hibernation or after exposure to low temperatures under natural conditions. In a series of experiments conducted in New Orleans from September to December, 1916, it has been found that the two common species of plasmodia, *Plasmodium vivax* and *Plasmodium falciparum*, in various stages of development in *Anopheles quadrimaculatus*, are able to survive exposure to lower degrees of temperature than 60° F. Furthermore, the results with *Plasmodium vivax* have shown that it may survive freezing temperatures of several days' duration,—a condition which rarely occurs naturally at this latitude.

In the present preliminary article the results which have furnished positive evidence of the resistance of *Plasmodium vivax* and *Plasmodium falciparum* to cold are presented. This evidence consists of the demonstration of the further development of oocysts and the viability of sporozoites subsequent to the exposure of infected mosquitoes to known and controlled temperatures varying from 60 to 29° F. and for periods of from 1 to 17 days.

The mosquitoes were bred specimens. They were infected by a single blood meal on suitable gamete carriers and were kept at room

temperature except for the periods of exposure. Controls from each lot of mosquitoes were kept under normal conditions for comparison with the experimental specimens.

The essential part of the data from experiments in which survival of the organisms was demonstrated is summarized in Table I.

TABLE I.

Experiment No.	No. of mosquitoes positive.	Time of development before exposure.	Temperature.	Duration of exposure.			Interval from exposure to dissection.	Evidence of resistance.			
								Subsequent development shown by.		Active sporozoites.	
								Oocysts.	Sporozoites in glands.	In oocysts.	In glands.
Resistance of *Plasmodium vivax* in *Anopheles quadrimaculatus*.											
		days	*°F.*	*days*	*hrs.*	*min.*	*days*				
2,026	5	8	51–69 Mean 59.3	6			4–11	5	2	2	
2,027	3	8	30–31		24		3–12	3	1	1	
2,028	5	8	40		24		2–19	5	2	2	1
2,029	3	8	35		24		8–12	3	2	1	
2,030	2	7	30		24	5	8	2		2	
2,031	2	7	29–30		48	5	9–15		2		2
2,032	2	7	31		96	10	7–14	2	1		
2,033	1	14	31		94	25	2			1	
2,035.2	1	18	38–59 Mean 46.1	15	19		8				1
2,035.3	1	17	45–51 Mean 48	6	18		0			1	
2,035.6	1	15	38–59 Mean 46.1	16	20		13				1
2,037	1	23	29–30		49	30	1			1	
Resistance of *Plasmodium falciparum* in *Anopheles quadrimaculatus*.											
2,018	1	12	35		24		6				1
2,019	3	12	54–57		48		1–8				3
2,022	2	13	55–57		24		4–16				2

In Experiments 2,026, 2,027, 2,028, and 2,029 the infected mosquitoes were exposed on the 8th day after the infective blood meal. The stage of development is indicated by the microphotograph reproduced in Fig. 1. The mosquitoes in Experiments 2,030, 2,031, and

2,032 were exposed on the 7th day (see Fig. 2), but owing to the lower temperature of the room during growth, the oocysts were much less developed than were those in the previous experiments. In each of these series the recovery of the parasites was demonstrated in later dissections by conclusive evidence of further development of the oocysts. Mature cysts (Figs. 3, 4, and 5), sometimes with active sporozoites, and empty capsules after the release of the sporozoites, were found. In a number of specimens the salivary glands had become infected with sporozoites (Figs. 6 and 7), and in a few activity of these was observed. The mosquitoes in the remaining experiments were exposed when the parasites were in later stages of development, and in these the evidence of recovery was based upon the later finding of active sporozoites either in the glands or in oocysts from which they had not yet escaped (Fig. 8). Other specimens were not included in the positive results, since activity of the sporozoites was not determined, although they appeared normal.

SUMMARY.

The experiments reported here have shown that the parasite of tertian malaria in the mosquito host is able to survive exposure to a temperature of 30° F. for a period of 2 days, 31° F. for 4 days, and a mean temperature of 46° F. for 17 days. In a smaller series of tests the sporonts of the estivo-autumnal parasite have shown a resistance to temperatures as low as 35° F. for 24 hours.

I am indebted to Dr. C. C. Bass and Dr. F. M. Johns, of the Tulane Medical Department, for suggestions and aid. The mosquitoes were bred for the experiments at the Mound Laboratory of the Bureau of Entomology, in charge of Mr. D. L. Van Dine.

EXPLANATION OF PLATES.

PLATE 38.

FIG. 1. 8 day old oocysts in a control specimen from the lot of mosquitoes used in Experiments 2,026, 2,027, 2,028, and 2,029. Sporoblast formation was not yet distinguishable. × 704.

FIG. 2. 6 day old oocyst in a control from the lot of mosquitoes used in Ex-

periments 2,030, 2,031, and 2,032. The exposure of the experimental mosquitoes was made a day later. × 704.

FIG. 3. Four oocysts in final stages of development from a specimen in Experiment 2,028. (Compare with Fig. 1.) × 704.

FIG. 4. An oocyst, with sporozoite development well advanced, from a specimen in Experiment 2,030. (Compare with Fig. 2.) × 704.

PLATE 39.

FIG. 5. Several mature oocysts, from a second specimen in Experiment 2,030. (Compare with Fig. 2.) × 704.

FIG. 6. Sporozoites from the salivary glands in a specimen from Experiment 2,031. (Compare with Fig. 2.) × 330.

FIG. 7. Sporozoites from the salivary glands in a specimen from Experiment 2,026. (Compare with Fig. 1.) × 330.

FIG. 8. An oocyst containing a mass of very active sporozoites 2 days after exposure to 31°F. for 94 hours, from a specimen in Experiment 2,033. × 704.

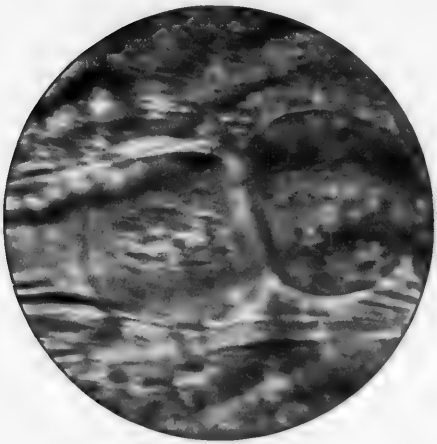

FIG. 1.

FIG. 2.

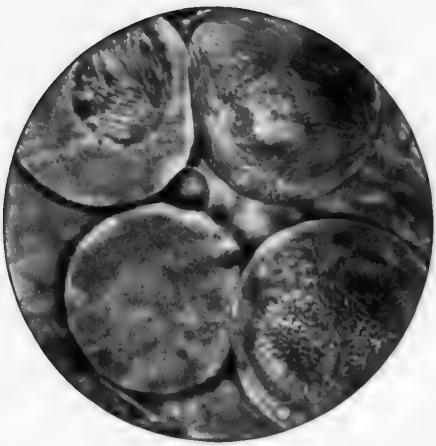

FIG. 3.

FIG. 4.

(King: Effect of Cold upon Malaria Parasites.)

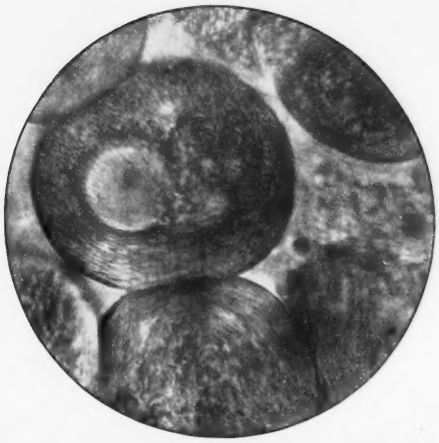

FIG. 5.

FIG. 6.

FIG. 7.

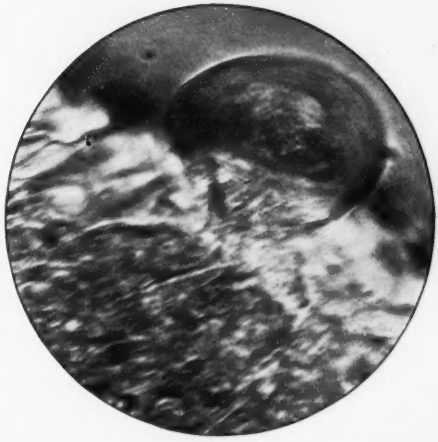

FIG. 8.

(King: Effect of Cold upon Malaria Parasites.)